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Review Article

The pathogenesis of endometrial polyps: a systematic semi-quantitative review

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Summary

The pathogenesis and natural history of endometrial polyps are not very clear. The objective of this study was to assess the opinions of international medical literature regarding the factors involved in the pathogenesis of endometrial polyps and to organize the results consistently with what is known about endometrial physiology. *Materials and Methods:* A systematic review was carried out with the following search engines: PubMed, OVID, Scopus, SCIELO, and AJOL. Two hundreds forty-six abstracts were selected from the literature; of these abstracts, 58 factors were extracted and set as causative, non-causative, unclear or protective link with endometrial polyps. This relation is described through a correspondence analysis and tested with a main effect hierarchical log-linear model. *Results:* The log-linear model resulted significant for the correspondence found with the following factors: (i) causative link (ageing, bcl-2 protein, excess weight/obesity, tamoxifen regardless of timing, relationship between estrogen receptors and progestinics, unbalanced estrogen therapy, estrogen-like effect, and unbalance between estrogens and progestinics), (ii) protective link (progestinics, antiestrogenic action), (iii) unclear link (menopause, ki-67 protein, angiogenesis, tamoxifen for a short time, tamoxifen for a long time, hormone replacement therapy (HRT), endometritis/inflammation), and (iv) non-causative link (none of the factors specifically). *Discussion:* Subsequently to a review of the physiology of the endometrium, the onsetting of endometrial polyps was suggested through estrogen-related and non-estrogen related ways; the two ways can overlap. The most implied factors in the development of endometrial polyps are linked with one of these or both ways.

Key words: Endometrial polyps; Pathogenesis; Estrogens; Progesterone; Apoptosis.

Introduction

Endometrial polyps are a very common pathology [1, 2]. They are easily treated through endoscopy [3], they can conceal tumors [4-6], and are often removed.

The endoscopic systematic removal of the polyps has contributed to an increase of publications of clinical studies focusing on risk or protecting factors and on some molecular aspects of pathogenesis. Notwithstanding the timing that polyps need to generate and why, should be taken into account to fully understand the pathogenesis. The time that polyps need to fully develop and their risk factors are unknown. In fact, only deWaay *et al*. [7] analysed the natural development of polyps. This study states that only the small polyps tend to spontaneously recede while the larger ones are more prone to continue growing. This suggests that some factors can play a beginning and promoting role for the polyps' development and these factors act in a synergic or sequential way.

To logically assess and order the vast literature on the endometrial polyps' pathogenesis, the authors undertook a systematic review with a semi-quantitative approach. This should assess the value that has been attributed to epidemiological or molecular risk factors in polyps' development.

Then, according to what is known about the endometrial physiology, the authors examined from a pathogenetic point of view, the most recognised risk factors.

Materials and Methods

On October 11th, 2011 a bibliographic research was carried out with the following search engines: PubMed, SCIELO, OVID, Scopus, and AJOL. The keywords used were the following: "endometrial polyps physiopathology", "endometrial polyps hormone", "endometrial polyps oxidative stress", "endometrial polyps growth factor", "endometrial polyps cytokines", "endometrial polyps inflammation", and "endometrial polyps risk factors".

The research obtained 1,067 references. From this list, duplicates, works of which abstracts were not relative to endometrial polyps, abstracts not directly or indirectly relative to endometrial polyp pathogenesis, and works without an abstract, resulted in a total residue of 246 references. These references are listed in Table 1.

These references' abstracts were read in order to extract any reference to endometrial polyp pathogenesis within the text. Such information is linked to one or more factors implied in endometrial polyp pathogenesis (e.g. tamoxifen use, hormonal unbalance, gene bcl-2 expression, etc.). Fifty-eight factors were identified through the abstracts; these are listed in Figures 1, 2, and 3.

The information can be extracted through logical links within the medical texts sentences [8] and have been used in the medical field to interpret biological processes [9]. Moreover, through this information, the competent readers can assess the relevance of the medical abstracts unbiasedly, similarly to how

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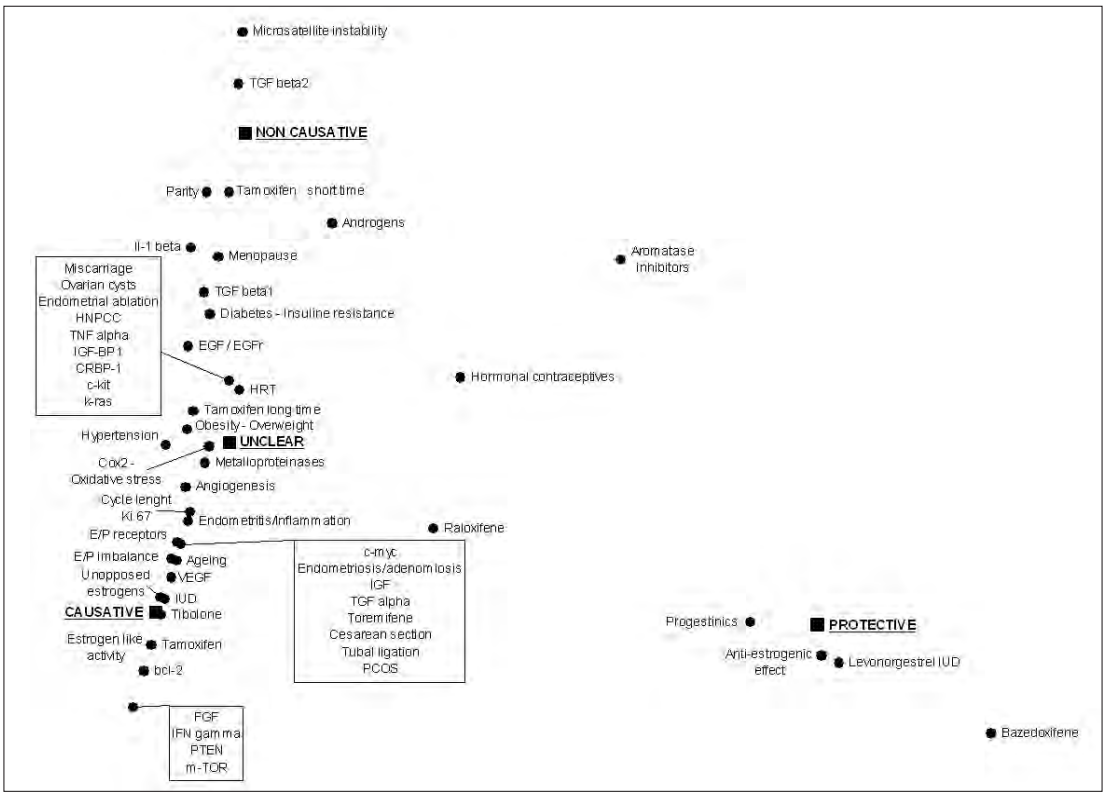

Figure 1. — Perceptual map from correspondence analysis. Causative link, non causative link, unclear link, and protective link are highlighted as the squared points of a bi-dimensional map. Fifty-eight factors are reported as smaller circular points. The closer the factors are to the squared points, the stronger the association is. E: Estrogens. P: Progesterone.
CRBP-1: Cellular Retinol Binding Protein-1. Cox-2: Cyclooxygenase-2. EGF: Epidermal Growth Factor. EGFr: Epidermal Growth Factor Receptor. FGF: Fibroblast Growth Factor. HNPCC: Human Non-Polyposis Colon Cancer. HRT: Hormonal Replacement Therapy. IFN: Interferon. IGF: Insuline Growth Factor. IGF-BP1: Insuline Growth Factor Binding Protein-1. Il-1: Interleuchin-1. IUD: Intra-uterine Device. PCOS: Polycystic Ovary Syndrome. TGF: Transforming Growth Factor. TNF: Tumor Necrosis Factor. VEGF: Vascular Endothelial Growth Factor.

mathematical algorithms would be used [10]. Therefore this information can be used for inference [8].

Based on the logical nexuses present in the sentences of the selected abstracts, every factor was assigned a causative link, a protective link, a non-causative link or an unclear link, with the development of an endometrial polyp.

The frequencies with which every factor was assigned a causative, protective, non-causative or unclear link are described through a two-dimensional correspondence analysis [11]. Such analysis allows to quantify the importance of the association of each factor with each of the four pathogenetic links just mentioned (causative, protective, non causative, and unclear). Such importance is evident in a two-dimensional representation (perceptual map).

The closer the factor is to one of the pathogenetic links and the more distant it is from the others (correspondence), the stronger the importance of the association is. Hence the distances between each factor and the predetermined logical links were calculated. Such distances were represented in a percentage scale, where the closest proximity corresponds to the highest importance according to international literature.

A log-linear model (main effect hierarchical log-linear model) was used to assess whether the correspondence found was significant. The standardized residuals extracted from the model have been graphically represented in order to highlight for which factors the log-linear model is relevant. The higher the standardized residuals are, the more it is agreed upon within the medical literature that the correspondence between that factor and that link is relevant. It is necessary to report the residuals because each factor's frequency relating to each pathogenetic link (causality, protection, indifference, and non-clarity) can bias the importance extracted from the correspondence analysis and represented through the percentage scale. For example, if a factor has been examined in just one study, the possible causative link expressed by the authors of that study in the abstract would result very strong in the correspondence analysis, even if there are no other opinions to support it. SPSS 16.0 was used for statistical analysis, with $p < 0.05$.

Results

In Figure 1 the authors show 58 factors that have been recognised as influencing polyp pathogenesis (perceptual map). After a log-linear analysis, the correspondence shown in Figure 1 appears statistically significant (likelihood ratio 752,154, $p < 0.001$; Pearson Chi-square 849,289, $p < 0.001$).

Figure 2 shows the importance each 58 factors that have been attributed according to the four pathogenetic links (causality, protection, indifference, and non-clarity).

In Figure 3 the highest value of standard residuals shows which factors have a stronger link with endometrial polyps. These factors included the following: ageing, menopause,

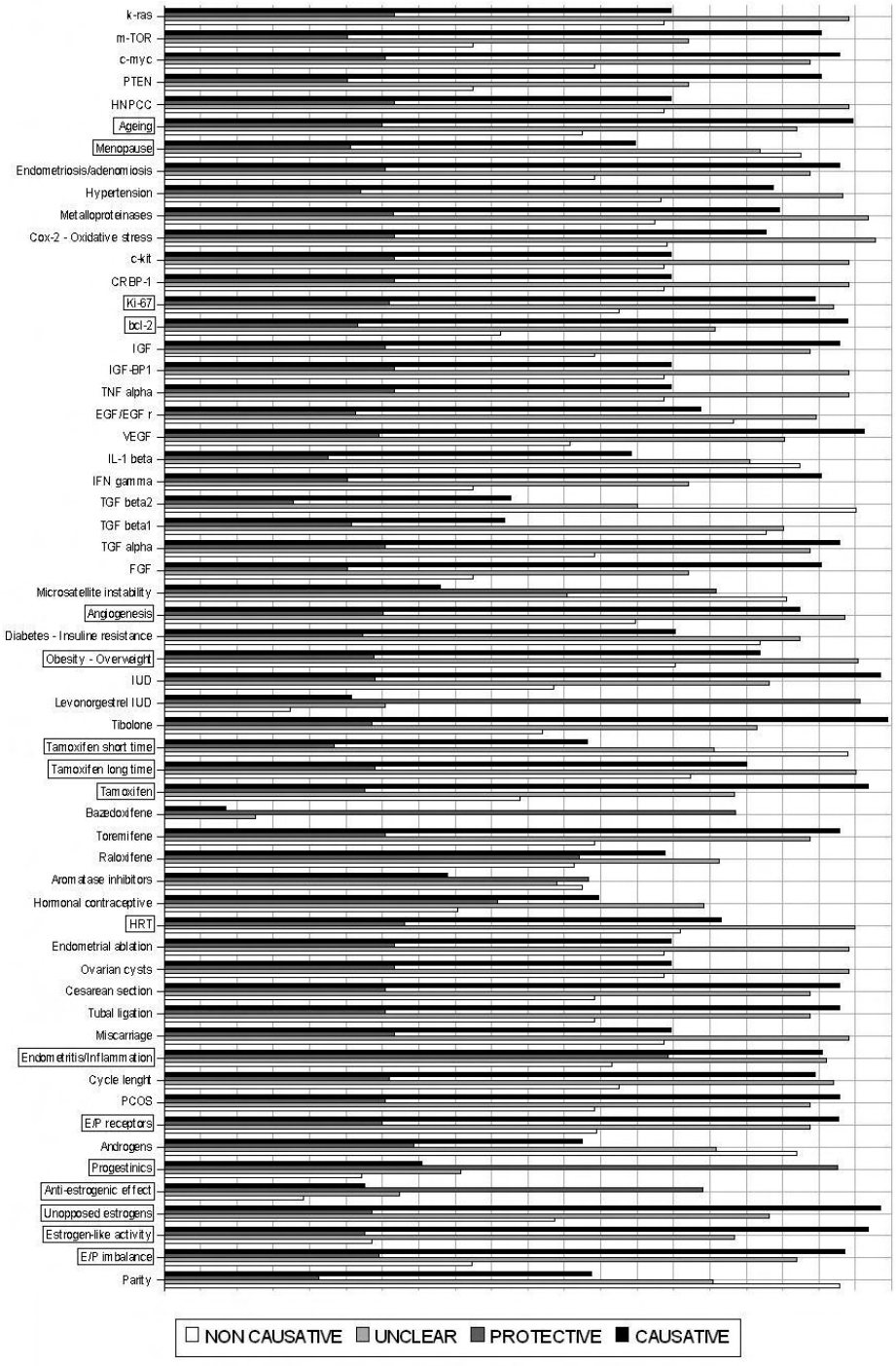

Figure 2. — Graphical representation of the relevance. It was calculated from distances of the perceptual map converted into a percentage scale. Factors more strongly associated with a link are highlighted in boxes.

protein ki-67, protein bcl-2, angiogenesis, obesity/overweight, tamoxifen (for a short time), tamoxifen (for a long time), tamoxifen (regardless of timing), HRT, endometritis/inflammation, relationship between estrogen receptors and progestinics, antiestrogen action, unbalanced estrogen therapy, estrogen-like effect, and unbalance between estrogens and progestinics.

These factors, according to the fixed link, can be ordered as follows: (i) causative link (ageing, bcl-2 protein, excess weight/obesity, tamoxifen regardless of timing, relationship between estrogen receptors and progestinics, unbalanced estrogen therapy, estrogen-like effect, and unbalanced between estrogens and progestinics), (ii) protective link (progestinics, antiestrogenic action),

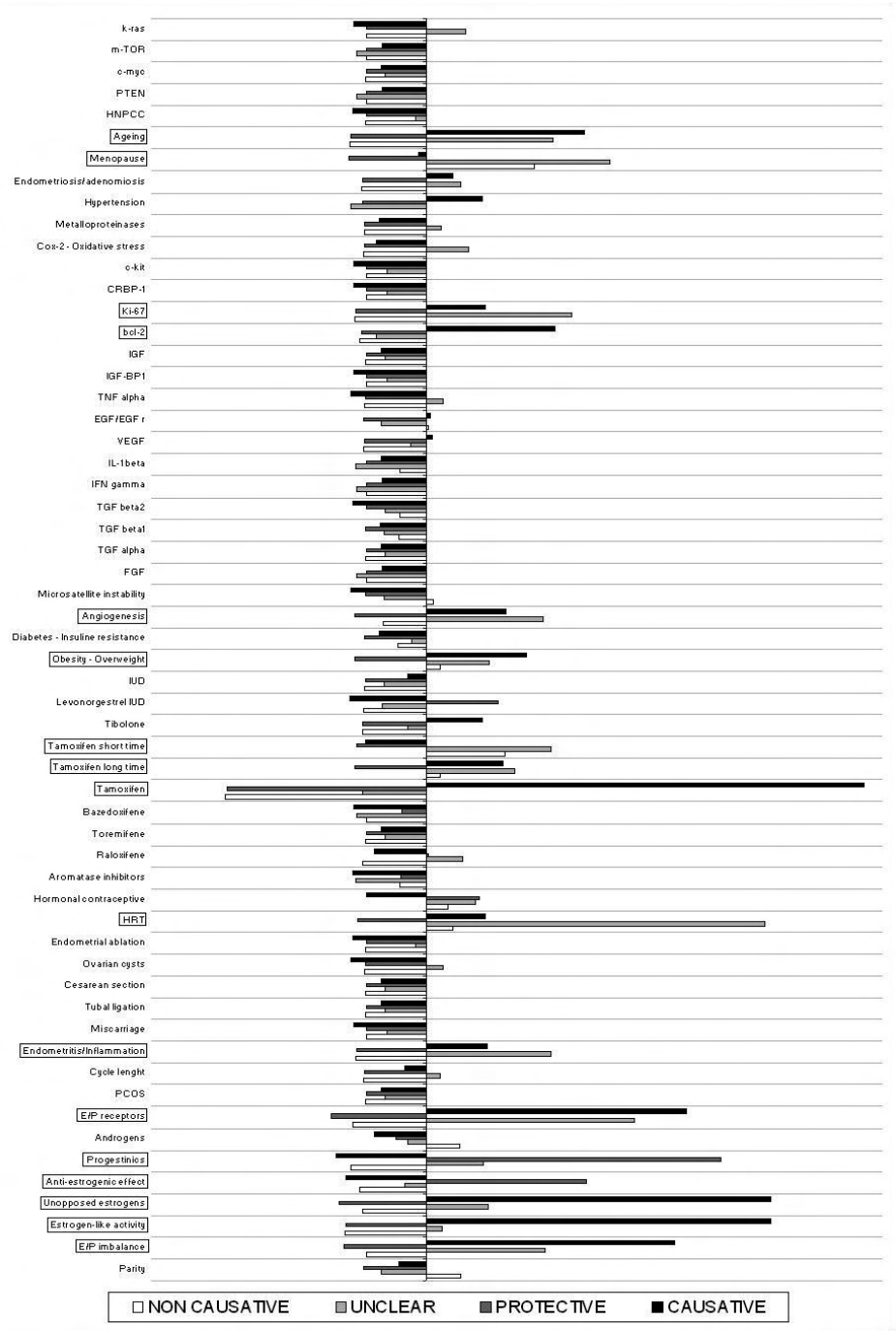

Figure 3. — Graphical representation of standardized residuals for each factors and for each link (causative, non causative, unclear, and protective). Factors with highest standardized residuals (both negative and positive) are considered to be more strongly associated with a link (causative, non causative, unclear, and protective) and are highlighted in boxes. The associative strength of a factor is in relation with the number of abstracts regarding that factor.

(iii) unclear link (menopause, ki-67 protein, angiogenesis, tamoxifen for a short time, tamoxifen for a long time, HRT, endometritis/inflammation), and (iv) non-causative link (none of the factors specifically).

Discussion

The statistical analysis run and the way the authors executed the systematic review (constrained to the availability of the search engines utilised) limit the reliability of the results in relation to the numerous parameters for which there are not many studies. Moreover, by only analysing the abstracts, the authors could not assess the opinions discussed in the full texts. These limitations, however, allow identifying with even more clarity which pathogenetic factors are more relevant as they are acknowledged in several abstracts. Therefore, according

to the results, the authors can conclude that the hormone hypothesis, which states that endometrial polyps develop because of an excess of estrogen activity, in relation to the progestogenic activity on the endometrium, is a widely-accepted hypothesis.

Other studies on the role of additional pathogenetic factors are not entirely conclusive. Some of these could be correlated to hormonal status, for example menopause [12, 13], obesity [14], the expression control of bcl-2 [15], while others cannot have attributed a clear pathogenetic identification (e.g. ageing). Moreover, the literature attributes an unclear link to some factors when their pathogenetic action is not explained even on a presumptive way.

According to what is known on the endometrium physiology, the next section will analyse the pathogenetic action of those factors that are believed to have a nexus with endometrial polyps, with the final aim of summarising in a unique model the endometrial polyps' pathogenesis.

Hormone hypothesis

Menstruation is the clinical expression of the cyclic growth and shedding of the endometrial lining under the control of hormones. Various microarray studies have highlighted how many sets of genes are expressed in the endometrium, during the transition between the advanced proliferative phase and the intermediate secretory phase [16], and how this does not occur in case of endometriosis [17]. The alpha and beta estrogen receptors are expressed in the nucleus of the glandular and stromal cells of the endometrium and tend to decline during the advanced secretory phase within the functional layer of the endometrium [18]. However in the stroma, beta receptor tends to decline less during the secretory phase [19]. For this reason, it is believed that the alpha receptor is the most responsible for the cyclic changes induced by estrogens in endometrium [20]. The expression of progesterone receptors is a consequence of endometrial exposure to estrogens during the first phase of the cycle. The estrogen alpha and progesterone receptors are upregulated during the secretory phase by progesterone [21]. The activation of estrogen beta receptor suppresses the expression of the alpha receptor [22, 23]. It is possible that estrogen alpha receptor may induce the expression of progesterone receptors in the stroma of the endometrium by binding to ovarian estradiol [24]. Finally, within the basal layer, progesterone receptors tend to be consistently expressed during the secretory phase, in the stroma, and close to the vasa [25].

The balance in the expression of estrogen and progesterone receptors at a functional and basal layer in the stroma and in the glandular epithelium regulates endometrial growth and its functions during the menstrual cycle. When an endometrial polyp forms, it is possible that limited areas of the endometrium are estrogen-hypersensitive; this could induce the endometrium to grow also during the second half of the menstrual cycle, thus promoting the growth of a polyp. As the endometrial polyp is formed of a stroma, in which a vascular axis coated in epithelial cells is found [26], the endometrial tissue growth must be specifically related to the endometrial stroma and the vasa, rather than to the epithelial lining. Ludwig *et al*. [27] report that during the proliferative phase, a transitory excess of growth of the endometrial tissue could take place, but the persistence of this during the secretory phase could result in micro polyps. This suggests a low sensitivity to progesterone for some areas of the endometrium. In medical literature, it is generally accepted that a progesterone stimulation of the endometrium or the exposition to substances with an anti-estrogen effect can prevent the onset of endometrial polyps (Figures 2 and 3). On the contrary, there does not seem to be a general consensus regarding the unbalance between estrogen and progesterone receptors as determinant in the development of an endometrial polyp (Figures 2 and 3). Immunohistochemistry studies that searched the link between hormone receptors and polyps in menstruating women have given contrasting results [28-33]. In summary, it seems that polyps in menstruating women are an expression of high levels of estrogen and progesterone receptors in the glandular epithelia, and low progesterone receptors in the stroma, with variable estrogen levels in the stroma.

Some authors [34, 35] have reported the presence of estrogen beta receptors in the nuclei of the vascular endothelial cells of the endometrium, suggesting a direct estrogen-angiogenic role through the beta receptor. As mentioned, the estrogen beta receptor is also the most present in the endothelial stroma during the menstrual cycle. If the estrogen beta receptors' response were to be higher than the alpha receptors, a reduction in the alpha receptor-mediated endometrial response would be expected during the proliferative phase of the menstrual cycle. This implies a reduction in the progesterone receptors' expression and a vascular endothelial and epithelial stroma growth where the estrogen beta receptor is largely expressed. This condition creates the prerequisites for the onset of one or more endometrial polyps. The results of Ye *et al*. [36] support the estrogen receptor imbalance assumption for polyp pathogenesis. These authors have demonstrated that, unlike the estrogen alpha receptor, the estrogen beta receptor is mostly expressed in the endometrial polyps' stroma, compared to a healthy endometrium, and that the extent of such expression is directly related to both serum and local estradiol levels.

Finally, a key point in endometrial polyps' development is the hyperactivation of the estrogen beta receptor, compared to the alpha, during the menstrual cycle's proliferative and secretory phases. This causes a growth of the endometrial stroma, angiogenesis, and progesterone insensitivity.

The role of apoptosis

The endometrial cycle ends with endometrial shedding. The shedding of the endometrial tissue in the basal layer is prevented during the advanced secretory phase by expression of the bcl-2, both on a stromal and on an

epithelial level [37, 38]. The bcl-2 gene expression allows the clonal expansion of the endometrial tissue and the development of a new functional level after endometrial shedding, with the beginning of a new endometrial cycle. Usually, the expression of some proteins of the blc-2 family immortalise the cells, preventing their apoptosis and it is regulated by various cytokines in inflammatory situations [39].

The hypothesis of a deficient apoptosis as a cause determining the onset of endometrial polyps is well-established within medical literature, especially when related to the gene expression for the bcl-2 protein (Figures 2 and 3). Taylor *et al*. [37] state that a pivotal point for the formation of an endometrial polyp is the loss of the regulation of the bcl-2 protein gene expression, concluding that the reason for the polyp's development is not related to an excess of endometrial growth but rather to a loss of physiological mechanisms of apoptosis. Other studies [15, 40-46] have been conducted in relation to the gene expression of bcl-2 when endometrial polyps occur, both in pre-menopause and post-menopause in patients under tamoxifen treatment, HRT, and tibolone therapy. The authors state that there is probably a hormonal implication in the control of the bcl-2 gene expression in endometrial polyps, both in pre- and post- menopause, although a strong quantitative relation between the hormonal receptors and the intensity of the bcl-2 expression has not been found.

The lack of association between hormonal receptors and bcl-2 expression can be explained because immunohistochemical studies provide semi-quantitative evaluations. Moreover, the way the gene expression of bcl-2 at the endometrial level is regulated is in essence not well-understood, although it is intuitive to believe that estrogen and progesterone can indirectly control expression [47]. Maia *et al*. [46] however, state that the bcl-2 gene expression is related to estrogen stimulation during the proliferative phase of the cycle.

It is then necessary to investigate the behaviour of bcl-2 gene when estrogen overstimulation occurs during the first half of the cycle, which continues then in the second half, according to the aforementioned hypothesis of unbalanced estrogen receptors. The patients with endometriosis provide a natural model of over-sensitivity to estrogen of this kind [48, 49]. These patients are over-sensitive to estrogen stimulation via beta receptors [50]. Moreover, the ectopic endometrium is unable to induce apoptosis because the bcl-2 gene is overexpressed. This overexpression is directly related to estrogen receptors [48]. Remarkably, even the eutopic endometrium of patients affected by endometriosis has a less-intense apoptotic capacity compared to the eutopic endometrium of healthy subjects [51]. Bulun [50] has stressed that eutopic endometrium of patients with endometriosis is able to over-express the cyclooxygenase-2 (cox-2), both constituently and via estrogen beta receptor. Cox-2, as known, is an inflammatory marker and induces cellular oxidative stress. Cox-2 expression at the endometrial level increases physiologically when progesterone decreases [52, 53], together with other inflammatory mediators [54-56]. This process has a role in promoting the regeneration of the endometrium after menstrual shedding. It is intuitive and also stated in medical literature [57] that oxidative stress can suppress apoptosis and this can occur also at the endometrial level.

Analysing the endometrium's behaviour in patients with endometriosis, it can be concluded that an inflammatory process is commenced by an excess of estrogen stimulation (via beta receptors) during the entire cycle and this can indirectly lead to inflammation and to the precocious overexpression of bcl-2 gene, via oxidative stress, of which cox-2 is a marker. This generates an exuberant growth of endometrium and angiogenesis and, in absence of apoptosis, endometrial polyps. Some other inflammatory scenarios, independently from hormonal stimulation, can nonetheless generate an endometrial polyp. The literature-established opinion and also deduced in this review support this hypothesis. It is recognised that inflammation can play a role in the generation of endometrial polyps (Figure 2) and there is anecdotal evidence (Figures 1 and 2), that shows a strong causality relation with situations where a stronger inflammation is possible or certain. For example endometrial polyps can be linked to a cesarean section scar [58], with an intrauterine device (IUD) not medicated with levonorgestrel [36, 59], with tubal ligation [60, 61], with endometritis [62], and as already mentioned, with endometriosis [63, 64].

Erdemoglu *et al*. [65], have analysed the cox-2 expression in endometrial polyps in patients during pre- and post-menopause, finding that cox-2 is more expressed in pre-menopause. This tends to be more prominent in epithelium, rather than in the stroma. It also suggests that cox-2 expression in polyps is under hormonal control, and this is indirectly confirmed by Maia *et al*. [66, 67].

The authors can conclude that the presence of an inflammatory state that stops apoptosis in the endometrial functional layer is very likely to be a pivotal point for endometrial polyps' onsetting. The endometrial inflammatory state can be found in various pro-inflammatory conditions (endometriosis/adenomyosis, endometritis, cesarean section scar, tubal ligation, IUD) and when there is an excess of estrogen stimulation. Moreover, given the large incidence of polyps in normal women, the excess of endometrial inflammation is likely to be caused by hormonal dysfunction. This is explainable considering that steroidal hormones normally regulate the mediators' expression of endometrial inflammation [68].

The role of growth factors

Epidermal growth factor/receptor (EGF/EGFr), transforming growth factor alpha (TGFalpha), and platelet-derived growth factor (PDGF) are mitogenic factors for the endometrium's basal layer cells, and are probably controlled by estrogens during the proliferative phase of the menstrual cycle in order to stimulate the endometrium's growth after its shedding [69]. Other cytokines are under progesterone control during the secretory phase, most likely in order to stop expression of metallopro-

teinase – responsible for menstrual shedding [70]. For example, TGFbeta1 suppresses the metalloproteinase at a stromal level [71].

Growth factors could play different roles in endometrial polyps' growth depending on the menstrual cycle's phase and on their localization. There is little evidence throughout international medical literature regarding growth factors' implications in endometrial polyps' growth. Maia *et al*. [72] suggested a role for EGF and for its receptor in both pre-menopausal and post-menopausal endometrial polyp growth, while Gray *et al*. [73, 74] have reported that diethylstilbestrol (DES)-induced TNFalpha's expression can aid in the growth of various types of uterine lesions in rats, including endometrial polyps. However, medical international literature tends to suggest that endometrial angiogenesis does have a role, to some extent, in the growth of endometrial polyps, although it is unclear in what way (Figures 2 and 3).

Physiologically, endometrial vasa growth is mostly controlled by vascular endothelial growth factors (VEGFs). Various isoforms of VEGFs are involved in normal endometrial vasa growth and seem to be constantly produced during the entire cycle [75-77] by the epithelium of the endometrium [78]. It is possible that a source of VEGFs that is important for endometrial vasa growth could be caused by the neutrophils adjacent to the endothelium [79]. Although VEGF's expression is not cyclical, Nayak and Brenner [80] have shown a cyclical growth of the endometrial vasa. This has led to believe that estrogens could induce cyclical expression of VEGF receptors half-way through the proliferative phase [80]. However many other vascular growth factors, including fibroblast growth factor (FGF) and TGFbeta1, control endometrial angiogenesis in a cyclical manner [81], most likely in relation with the hormonal state. It is thus probable that many of these angiogenic growth factors could be under ovarian steroid direct and indirect control, in a not entirely clear way.

Regarding endometrial polyps, Xuebing *et al*. [82] reported that TGFbeta1 is, together with VEGF, involved in their growth, while Hague *et al*. [83] reported that both acid and basic FGF and adrenomedullin are mostly present in the endometria of pre-menopausal women following tamoxifen treatment, suggesting a role for angiogenesis in the pathogenesis of endometrial cancer and endometrial polyps. Cheng *et al*. [84] reported that vasa density and VEGF expression are higher in endometrial polyps compared to healthy endometria, suggesting a physiopathological link between angiogenesis and the growth of endometrial polyps. Maia *et al*. [67] also report a role for VEGF in the development of various uterine pathologies, including endometrial polyps.

Therefore it is believed that a third pivotal reason for which an endometrial polyp develops is the expression of various angiogenic growth factors under hormonal control. The specific role of each of these growth factors in polyps' pathogenesis is yet to be determined, considering the role that the control of cellular apoptosis could play within the growth factors [85].

The role of metabolism and ageing

A specific discussion should be dedicated to the Insulin Growth Factor-I (IGF-I) and to the Insulin Growth Factor Binding Proteins (IGFBPs). Estrogens' control IGF-I during the proliferative phase of the cycle, while during the secretory phase its action is limited by the expression of IGFBP-1 and 3 [86], which sequestrate it and impede its biological action via IGF receptor. Rutanen *et al*. [87, 88] stated that the systemic and endometrial deregulation of IGFs/IGFBPs system can also lead to the onset of malignant and benign endometrial diseases, i.e. endometrial polyps, and the systemic and local deregulation of the IGFs/IGFBPs system can also be determined by metabolic disorders [88, 89]. Ben-Nagi *et al*. [90] have shown a reduction of IGFBP-1 expression in the secretory phase in patients with endometrial polyps. This determines an increase of IGF-I availability for its receptor. IGF-1 regulates apoptosis in various layers controlling the bcl-2 gene expression among others [91]. Keeping in mind that the interruption of apoptosis is considered fundamental in the onset of endometrial polyps, a certain number of polyps can be generated by inhibition of IGF-I mediated apoptosis, explaining why endometrial polyps are related to basal glucose levels [63], diabetes [13], with body mass index (BMI) [92], and arterial hypertension [12, 13, 63]. The regulation of IGFs/IGFBPs could be independent from estrogen activity. Belisario *et al*. [93] stated that polyps' growth is independent from the expression of estrogen receptors in menopausal patients with a high BMI. Therefore, according to the evidence in medical literature, endometrial polyps are, in a percentage of cases, likely to be related to metabolic problems that affect the IGFs/IGFBPs.

Strong evidence in literature is found regarding the link between elderly patients and endometrial polyps (Figures 2 and 3). This relation is independent from menopause, which does not appear as a risk factor in the multivariate analyses [63, 94, 95]. Although in menopause hormonal production is reduced, molecular biological studies have confirmed that even during menopause, endometrial polyps are related to the expression of hormonal receptors [29, 30, 42, 96, 97]. The endometrial polyp's growth in post-menopausal patients is likely to depend on an unbalanced estrogen and progesterone receptor response in some areas of the endometrium. Gul *et al*. [29] have shown that there is a negative correlation between receptors for stromal progesterone and patients' age. Moreover, older studies on animal models and humans [98-101] stated that, when ageing, the diffusion of hormonal receptors for estrogen is more variable within endometrial stroma rather than in the epithelium, and therefore the response to estrogen stimulation is more variable in older animals or humans. Kenemans *et al*. [102] hypothesized that by exposing the endometrium to pulsed estradiol, it is possible to influence the relative abundance of hormonal receptors, with a consequent up-regulation and selective activation of beta receptors. If we embrace this hypothesis, we can expect that during menopause, endometrial polyps can arise from some areas of the endometrium, which are irregularly sensitive to estrogens, with minimal

estrogen hormonal stimulation via beta receptors. Moreover, these polyps in post-menopausal women show deregulation in apoptosis mechanism as they do in pre-menopausal women [15]. Growth factors can also play a role in polyps' development in post-menopause. Loverro *et al*. [103] have shown that TGF beta1 is more expressed in atrophic endometria compared to proliferative and secretory phases.

To conclude, endometrial polyps in post-menopausal patients are likely to develop in a similar way as in pre-menopausal women, taking into account that older patients' sensitivity to estrogen might result in being a lot less predictable compared to pre-menopausal women. The different prognostic relevance of metabolic disorders, suggested by obesity, hypertension, and diabetes, might explain a higher number of polyps in older patients and the oncological implications that polyps have in post-menopausal patients [4, 5, 104].

The role of Selective Estrogen Receptor Modulators (SERMs)

There is large consensus among medical international literature that tamoxifen is determinant in the onset of endometrial polyps (Figures 1, 2, and 3). However, it is still unknown how long it takes for tamoxifen to generate a polyp. In fact, it is still unclear if taking tamoxifen for several months is less dangerous than taking it for years. Few studies have focused on other SERMs with regards to polyps' development. Pinkerton *et al*. [105] have shown that bazedoxifene has a protective effect on the endometrium, as it does not increase the risk of developing polyps when compared to placebo. It has been stated that raloxifene can have a protective effect on the endometrium during menopause [106], while other authors have identified ovarian activation signs in pre-menopausal patients treated with raloxifene, which can lead to the generation of polyps [107]. Finally Zhou *et al*. [108] have shown a similar action spectrum of toremifene to tamoxifen, with the chance of it to lead to endometrial polyps.

Despite the vast literature focusing especially on tamoxifen, it has not been explained in a conclusive way how SERMs can cause the onset of endometrial polyps. Many authors believe that the estrogen effect similar to tamoxifen is responsible for endometrial polyps' development. SERMs' effect on estrogen receptors is variable within different tissues [109, 110]. SERMs' behaviour on the endometrium is presented in the comprehensive review by Cano & Hermenegildo [111]. The biological consequences of stimulation with SERMs on the endometrium will vary depending on the type of SERM, on SERM's metabolism, and on the relative quantity of circulating estrogens and endometrial estrogen receptors. Furthermore, the relative expression of estrogen receptors depends on various factors, such as dysfunctional cycles, hyperestrogenism, previous hormone therapies, inflammatory processes, and ageing. Tamoxifen, and other SERMs likewise, has an influence on the expression of hormonal receptors in a healthy endometrium in post-menopausal women, increasing the quantity of glandular estrogen and stromal progesterone receptors [112, 113]; this effect also occurs in pre-menopausal patients [114]. The estrogen alpha receptor seems to be more expressed than the beta receptor within glandular and stromal epithelia in ovariectomized monkeys treated with tamoxifen in basal and functional layers of the endometria [115]. Tregón *et al*. [116] have shown an immediate increase of ki-67 expression and estrogen receptors after a 21-day tamoxifen treatment, finding simple endometrial hyperplasia through histological examination. Similar results have been revealed by Karack *et al*. [117] for progesterone receptors when treated with tamoxifen. At a later stage, however, the histological effect of endometrial stimulation for patients treated with tamoxifen is variable and seems to be independent from the expression of estrogen receptors [118, 119]. Wang *et al*. [115] hypothesised that during tamoxifen treatment, the activation of the estrogen beta receptor inhibits the expression of the alpha receptor. Therefore the protective effect of tamoxifen on the endometrium could through an estrogen-like activity be more active on the beta receptor when the ERalpha/ERbeta ratio is low. This, as mentioned above, might contribute to the onset of endometrial polyps.

As in a healthy endometrium, also in endometrial polyps of women treated with tamoxifen, there seems to be a variable effect on the expression of estrogen receptors. Dibi *et al*. [120] state that endometrial polyps in women treated with tamoxifen can or cannot express estrogen receptors, and those that do not express these receptors are more frequent when the endometrium is atrophic. Both the estrogen alpha and beta receptors are expressed in endometrial polyps in patients treated with tamoxifen [121]. Schwartz *et al*. [119] also found significant differences in estrogen receptor expression in tamoxifen-related polyps (low stromal levels) compared to non-tamoxifen related polyps (high stromal levels). On the other hand, McGurgan *et al*. [40] have shown that polyps in menopausal women treated with tamoxifen demonstrate a higher amount of progesterone receptors, a higher quantity of bcl-2, and less estrogen receptors, and they have also attributed the most important pathogenetic role for the development of endometrial polyps for patients treated with tamoxifen to apoptosis. As endometrial polyps in patients under tamoxifen treatment do not present high estrogen receptors levels, the assumption of an estrogen-like effect of tamoxifen as a cause of endometrial polyps could not always be valid. Such a possibility is consistent with what has been reported by Cano and Hermenegildo [111] as they highlight how a light tamoxifen stimulation profile on the endometrium could be present regardless of the estrogen-like action. In fact the anti-estrogen effect on the endometrium of tamoxifen increases with time [118, 119], most likely via beta receptors [115]. An unbalanced estrogen-like effect over alpha and beta estrogen receptors is therefore more likely at the beginning of treatment with tamoxifen. As treatment continues, tamoxifen could allow an endometrial polyp to grow by favouring estrogen-independent ways, involving agiogenesis, non estrogen-related apoptosis block, and

cellular proliferation. In fact FGF [83], adrenomedullin [83, 122], and TGFalpha growth factors, TNF-II (113) and EGF receptors, and expression of the ki67 antigene [114, 116], have been higher during tamoxifen treatment. On the contrary, IGFBP-1 is less-expressed during tamoxifen treatment [123].

Concluding, tamoxifen (and most likely other SERMs as well), are able to allow polyps to form probably via two ways: the first is estrogen-related and probably depends very much on the individual endometrial hormonal receptor expression, before and during tamoxifen treatment; the second way is non estrogen-related, that carries angiogenesis, cellular growth, and apoptosis inhibition through unknown mechanisms. This interpretation is justified by the fact that there is no unanimous opinion on how long tamoxifen needs to form endometrial polyps (Figures 2 and 3).

Moreover, the non estrogen-related polyps' oncological meaning during tamoxifen treatment could be different compared to the estrogen-related polyps.

The role of hormone replacement therapy (HRT)

This review's results do not give HRT a clear role in polyps' development. Many studies' abstracts express a pathogenetic role for HRT in endometrial polyps' development. Probably, HRT effect on the endometrium depends on how the hormones are administered [124], on their quantity [125, 126], and on the administration scheme [127]. Moreover, the number of endometrial hormone receptors is variable in post-menopausal women due to the ageing process, so the effect of the same type of HRT should be variable. Hanifi-Moghaddam *et al*. [128] considered the expression of certain sets of genes in endometria in healthy menopausal women under estradiol, tibolone, and estradiol plus medroxyprogesterone acetate treatment over 21 days. Endometrial genes' profile expressed during a balanced hormonal therapy is more similar to the results when no treatment is being administered. On the contrary, gene expression facilitated by tibolone appears to be more similar to that of estradiol, although the expression of some genes is specific only to tibolone. The same authors [129] highlighted how cellular proliferation and stromal and endometrial glandular epithelia apoptosis are at their highest during estradiol treatment, at a medium level during either tibolone or balanced estroprogestinic treatment, and at their lowest when no treatment is being administered. During hormonal treatment, IGFBP-3 is less-expressed, while IGF-I is more expressed. The effect of hormonal treatment on the expression of estrogen receptors (alpha and beta) and for progesterone, seems to be less intense [128]. These data lead to believe that a balanced HRT could be more protective for the endometrium compared to a therapy with just estrogens or tibolone.

Regarding endometrial polyps, McGurgan *et al*. proved that HRT [41] does not influence estrogen and progesterone receptors' expression, and tends to increase bcl-2 levels while inhibiting apoptosis. On the contrary, Maia *et al*. [130] reported that bcl-2 protein and and ki-67 anti-

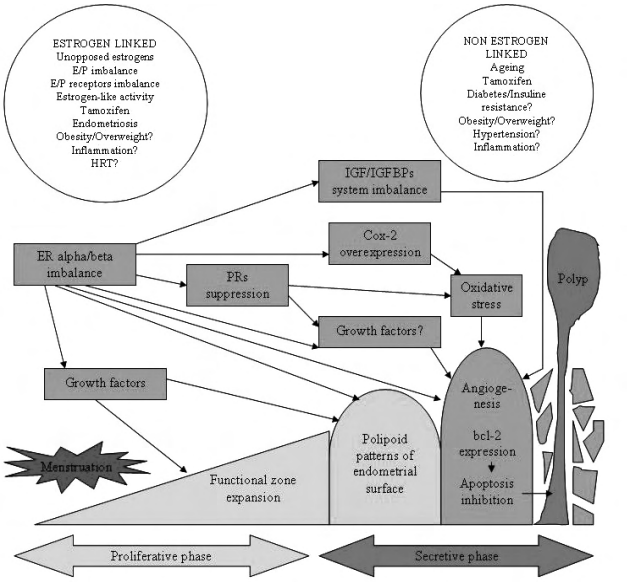

Figure 4. — Proposed model of endometrial polyps pathogenesis. Estrogens may lead to overgrowth of endometrial functional zone via estrogen beta receptor during the proliferative and early secretive phases. This behaviour may trigger oxidative stress via cox-2 and angiogenesis. Angiogenesis is promoted via endothelial estrogen beta receptor and through growth factors in the late secretive phase. When bcl-2 was expressed in the functional zone in the late secretive phase, inhibition of apoptosis saved some islets of endometrium from menstrual breakdown with the onset of polyps. In the succeeding endometrial cycle, polyps may grow in relation to estrogens' sensitivity of both glandular epithelium and stroma and vascular endothelium. Stromal overgrowth could be mainly linked to estrogen beta receptor sensitivity, while glandular epithelium overgrowth could be mainly linked to estrogen alpha receptor sensitivity. Therefore, estrogen-linked endometrial polyps should have a limited growth potential, and their clinical behaviour is set off by hormonal receptors' expression.
Moreover, the IGFs/IGFBPs may have a role in developing endometrial polyps, favouring or causing the onset of polyps independently from hormonal status and hormonal sensitivity. It is unclear if other kinds of growth factors may be able to favour the onset and growth of endometrial polyps independently from hormonal status. The authors suggest to portray this kind of polyp as non estrogen-linked. Some factors may favour polyp onset and growth with both hormonal triggers and non-hormonal triggers. For example, in tamoxifen-linked polyps, the angiogenesis, apoptotis inhibition, and growth of the polyps could be linked both to growth factor/receptor pathways and to estrogen receptor pathway.
Therefore, a double way of endometrial polyps formation is proposed: the most important is the estrogen-related way. Factors mostly involved in this way are listed in the upper-left circle of the Figure. Ageing, metabolic syndrome, and SERMs therapy, may cause endometrial polyp formation through unknown mechanisms. This quote of non-estrogen-linked polyps could have a growth potential and malignant potential differing from the estrogen-linked ones. Factors mostly involved in this way are listed in the upper-right circle of the Figure. Obviously, the estrogen-related and non-estrogen-related ways may overlap.

gene are less-expressed in polyps in women under HRT, concluding that it would allow polyps to grow regardless of bcl-2 and ki-67. Maia *et al*. [131] have also reported that polyps in menopausal patients can express estrogen receptors but are not sensitive to HRT with progestin. These clashing data lead to believe that HRT has a variable effect on the endometrium, sometimes independent of the endometrial hormone sensitivity. In fact if the endometrium was sensitive enough to HRT progesterone, it would be possible to speculate about the balanced HRT as able to prevent the growth of endometrial polyps, in agreement with what international medical literature states regarding progestins (Figures 2 and 3). However, as HRT also has an effect on the IGFs/IGFBPs system [128], it is possible that there is a non-estrogen-related developmental way for endometrial polyps during HRT. In this case as well, endometrial polyps could have a different oncologic potential to those non-estrogen-related.

The role of ki-67

The ki-67 protein is a cellular proliferation indicator [132]. The expression of the ki-67 protein is in an inverse relation with apoptosis in different types of cancer [133, 134]. During the proliferative phase, ki-67 is expressed in the endometrium under the control of estrogen [32], and progestin treatment will reduce its expression [135].

Some studies have considered ki-67 protein's expression in relation to endometrial polyps [15, 37, 40-44, 46, 66, 130, 136]. These studies aimed to assess cellular proliferation through ki-67, in relation to bcl-2 mediated apoptosis in pre-menopausal patients, in post-menopausal patients, during HRT, and during treatment with tibolone or tamoxifen. The results support the assumption of an apoptosis deficit rather than of a direct polyp growth. However an endometrial polyp is not a tumor lesion, that is able to grow independently and freely, in which ki-67 is overexpressed [134]. It is thus explainable how the pathogenic link between ki-67 and polyps, which is probably indirect, remains unclear.

Conclusions

In light of international medical literature's opinion and of the endometrium's physiology, it is concluded that endometrial polyps in most cases arise because of estrogen hypersensitivity in some areas of the endometrium, probably caused by a hyper-activation of the beta estrogen receptor on the alpha receptor during the first phase of the cycle or in post-menopausal women. Furthermore, they do not shed with menstruation because the estrogen-related inflammation could block apoptosis via bcl-2 gene expression (oxidative stress induction, cytokine production). This estrogen-related polyp growth could occur due to angiogenic growth factors' deregulation, produced under hormone control inside the polyp, within a short time, and few cycles. This interpretation explains why small polyps tend to regress, while large ones tend to develop and persist in time [7], and why polyps are clonal lesions [137, 138].

Apoptosis' via bcl-2 control and some endometrial polyps' proliferation, especially if in an elderly patient, during an inflammation or metabolic syndrome, could be independent from estrogens. These endometrial non-estrogen-related polyps could have a different behaviour and neoplastic potential compared to estrogen-related polyps. Moreover, these non-estrogen-related polyps would still be sensitive to hormones whose effect could amplify their growth and neoplastic potential.

This interpretation is summarized in Figure 4 and organizes the vast medical literature regarding endometrial polyps, hoping to guide future studies on their pathogenesis and prognosis in a clinically useful way.

Table 1. — *List of references used for semi-quantitative analysis*

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Original Articles

Frequency and risk factors of lower limb lymphedema following lymphadenectomy in patients with gynecological malignancies

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Summary

Objective: Lower limb lymphedema (LLL) is a major cause of morbidity in patients with gynecological malignancies after surgical treatment involving lymph node (LN) dissection. The aim of this study was to estimate the prevalence of LLL in such patients and detect risk factors for its occurrence. *Materials and Methods:* A retrospective analysis of all patients undergoing lymphadenectomy in newly-diagnosed gynecological malignancies at the University Hospital of Zurich between 2000 and 2007 was performed. Data from 313 patients were collected. Twenty patients with pre-existing edema or missing information were excluded before analysis. Time-to-LLL was estimated using the Kaplan-Meier estimate and potential risk factors were evaluated by a Cox regression model. *Results:* Estimated prevalence of LLL one year after surgery was 32%, increasing to 58% eight years after surgery. Median time to diagnosis of LLL was 5.2 years. The number of removed lymph nodes was significantly associated with time-to-LLL. Diagnosis of postoperative lymphocysts and local infections were accompanied by a significantly elevated risk for the development of LLL. Furthermore, time-to-LLL decreased with a higher body mass index (BMI) of the patient. In contrast, chemo- and radiotherapy, age, positive LNs, site of lymphadenectomy, and type of cancer were not observed to be associated with the occurrence of LLL. *Conclusions:* LLL is a frequent postoperative complication in patients undergoing lymphadenectomy for gynecological malignancies. It is thus imperative to sufficiently educate patients about the risk and symptoms of LLL prior to surgery. The data clearly show an association between time-to-LLL and number of dissected LNs, stressing the need to prospectively analyze the prevalence of LLL and carefully plan LN sampling as increasing knowledge is gained regarding the therapeutic benefit of sentinel and systemic lymphadenectomy in patients with different stages of gynecological malignancies.

Key words: Lymphedema; Gynecological malignancies; Risk factors.

Introduction

Secondary lymphedema is a major complication and source of morbidity after dissection of lymph nodes (LNs) in patients with gynecological malignancies. Diagnosis of lower limb lymphedema (LLL) is difficult and lymphedema is frequently overlooked by the physician if it is not reported by the patient [1-3]. Lymphedema initially presents with changes in appearance or sensation of the affected region, including swelling, visible lumps, puffiness and reddened areas, as well as pain, heaviness, hardness, heat, tenderness, and sensation of pins and needles [1]. Symptoms are often noticed within the first 12 months after surgery [3, 4]. With chronicity frequently due to delayed diagnosis or treatment and accompanied by tissue remodelling, the involved structures develop the characteristic features of induration and fibrosis [5]. The consequences are a deterioration of the quality of the patient's life with important health, as well as financial implications, leading to a considerable economic burden to healthcare systems [1, 3, 6-8].

Reported prevalence of LLL following surgery for gynecological malignancies ranges from 2.1% to 44% [3, 4, 6, 7, 9-15]. Lymphadenectomy is employed for staging and improvement of prognosis in nodal positive patients. A large number of LNs is frequently removed to obtain a reliable nodal status; however, this in turn may impair lymphatic drainage.

The number of removed LNs has repeatedly shown to have a significant impact on the development of LLL [4, 9, 10]. Other potential risk factors include radiotherapy [2, 4, 11], postoperative infection [16], and removal of LNs in the groin region [4]. Various authors previously excluded chemotherapy [4, 11], body mass index (BMI) [4, 9], positive LNs [2, 12], and age [4, 9, 12] as contributing factors. However, the pathophysiology of LLL is still not fully understood and to date, there are no reliable guidelines available permitting the clinician to identify patients at high-risk to develop LLL.

The present study determined the occurrence of LLL in patients undergoing lymphadenectomy in newly diagnosed gynecological malignancies and identified associated risk factors.

Materials and Methods

Patients

A retrospective analysis of all patients undergoing lymphadenectomy in newly diagnosed gynecological malignancies at the University Hospital of Zurich between 2000 and 2007

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was performed. Of the 313 patients for whom data were collected, two patients with incomplete records and 18 patients with pre-existing edema were excluded from further analysis. According to the type of cancer, a distinction was drawn between cervical (74 patients), uterine (104 patients), ovarian (71, including five patients with cancer of the tube), vulvar and vaginal (31 and four patients, respectively), and dual primary cancers (eight patients with uterine and ovarian cancer, one patient with uterine and cervical cancer). In respect to the site of lymphadenectomy, pelvic only, para-aortic, and inguinofemoral LN dissections were differentiated. Most patients with para-aortic lymphadenectomy also underwent pelvic lymphadenectomy. Positive LNs were documented for each patient. Depending on the type and stage of cancer, patients underwent standard local surgery with or without adjuvant therapy. Patients undergoing radiotherapy received either intravaginal brachytherapy of 20-25 Gy, external beam radiotherapy of 20-95 Gy, or a combination of both with cumulative doses of 50-122 Gy. Standard chemotherapy in patients with uterine or ovarian cancer consisted mainly of paclitaxel and carboplatin. Cisplatin was predominantly administered to patients with vulvar/vaginal or cervical cancer (Table 1).

Clinical information including age and BMI at diagnosis, type of cancer, number and site of dissected LNs, occurrence of postoperative infection and lymphocyst formation, radiotherapy, and chemotherapy were abstracted from patients' medical records. All patients included in this study underwent a postoperative physical examination performed by a gynecological oncologist. Postoperative infection was defined as documented erythema or inflammation surrounding the wound and requiring antibiotic therapy, abscess formation, infected lymphocyst or infected hematoma. Lymphocyst formation was verified by ultrasound and/or computed axial tomography. Patients with LLL were identified through a review of all available medical records, as documented by the physician and/or self-reported by the patient. Patients with cases of edema exclusively attributable to other causes such as renal, cardiovascular, or thrombotic disease, were considered non-lymphedema patients. Symptomatic lymphedema were subdivided into mild (lymphedema of the mons pubis, discrete LLL), moderate (LLL requiring therapy such as compression stockings or manual lymph drainage), and severe (therapy resistant LLL or causing serious complications e.g. erysipelas). Median patient follow-up period after initial surgery was 24 months (CI [16, 30]). A total of 293 patients entered the analysis for evaluation of potential risk factors associated with the occurrence of LLL.

Statistical analysis

Descriptive statistics include frequencies and percentages for categorical and median (range) for continuous data. The main endpoint was time-to-LLL, defined as the time between surgery and diagnosis of LLL. If a patient was seen without LLL, she was censored at the last follow-up date. Time to mild, moderate, and severe edema was handled in a similar manner; the authors censored all patients not seen with the edema type of interest for a given endpoint. Survival functions were estimated using the Kaplan-Meier estimate to report the probabilities of LLL. Time-to-LLL was estimated using the inverted Kaplan-Meier estimate. To obtain confidence intervals for a survival curve after a given follow-up time, Peto's variance estimate was used. A Cox regression model was generated to associate potential risk factors to time-to-LLL. To obtain a reduced model, stepwise variable selection was performed according to the Bayesian Information Criterion (BIC).

The authors did not perform a correction for multiple testing in these exploratory analyses. All tests were performed at a significance level of $p = 0.05$ and confidence intervals were computed at a confidence level of 95%.

Statistical analysis was conducted using R software [17]. Kaplan-Meier estimates and Cox regression were performed with the R-package survival [18]. Stepwise variable selection via BIC was performed employing the function stepAIC from the R library MASS [19].

Table 1. — *Treatment characteristics according to the type of gynecological cancer.*

	Type of gynecological cancer n (%)				
	Cervical	Uterine	Ovarian	Vulvar/ Vaginal	Dual cancer
All	74 (25.3)	104 (35.5)	71 (24.2)	35 (11.9)	9 (3.1)
Radiotherapy Yes	44 (59.5)	66 (63.5)	1 (1.4)	13 (37.1)	5 (55.6)
Chemotherapy Yes	37 (50.0)	13 (12.5)	55 (77.5)	6 (17.1)	7 (77.8)
Site of lymphadenectomy					
Pelvic only	53 (71.6)	65 (62.5)	18 (25.4)	2 (5.7)	2 (22.2)
Para-aortic	21 (28.4)	39 (37.5)	53 (74.7)	1 (2.9)	7 (77.8)
Inguinofemoral	1 (1.4)	1 (1.0)	0 (0.0)	32 (91.4)	0 (0.0)
Positive lymph nodes	23 (31.1)	23 (22.1)	22 (31.0)	14 (40.0)	0 (0.0)

Table 2. — *Clinical characteristics and estimated probabilities of LLL.*

Characteristics	n (%) of patients	Rates of LLL 1 year after surgery in % [95% CI]	Rates of LLL 8 years after surgery in % [95% CI]
All	293 (100)	32 [26; 38]	58 [47; 67]
Mild LLL	40 (13.7)	14 [9; 19]	29 [18; 38]
Moderate LLL	45 (15.4)	15 [10; 20]	34 [22; 45]
Severe LLL	16 (5.5)	8 [4; 11]	11 [4; 17]
Type of gynecological cancer			
Cervical	74 (25.3)	28 [15; 39]	45 [20; 62]
Uterine	104 (35.5)	31 [20; 41]	67 [44; 80]
Ovarian	71 (24.2)	26 [14; 37]	52 [30; 67]
Vulvar/Vaginal	35 (11.9)	60 [32; 77]	70 [35; 86]
Double primary cancer	9 (3.1)	35 [0; 61]	68 [3; 89]
Site of lymphadenectomy			
Pelvic only	90 (30.7)	22 [14; 30]	52 [36; 65]
Para-aortic	121 (41.3)	37 [27; 46]	59 [41; 71]
Inguinofemoral	34 (11.6)	62 [33; 79]	75 [32; 91]
Radiotherapy			
Yes	129 (44.0)	28 [19; 36]	52 [36; 64]
No	164 (56.0)	36 [27; 45]	63 [46; 75]
Chemotherapy			
Yes	118 (40.3)	27 [18;35]	54 [38; 65]
No	175 (59.7)	36 [27;44]	60 [44; 72]
Postoperative infection			
Yes	42 (14.3)	50 [30; 64]	73 [33; 89]
No	251 (85.7)	29 [22; 35]	55 [43; 64]
Postoperative lymphocyst			
Yes	61 (20.8)	53 [37; 64]	75 [49; 88]
No	232 (79.2)	27 [20; 33]	53 [40; 63]

LLL: Lower Limb Lymphedema; CI: Confidence Interval.

Table 3. — *Patient characteristics (continuous variables).*

	Minimum	Median	Maximum	IQR
Number of removed LNs	1	27.00	97	21.00
BMI	15.4	24.70	50.00	7.10
Age	24.00	57.00	89.00	20.00

LN: Lymph Node, SD: Standard Deviation, IQR: Interquartile Range.

Results

Estimated prevalence of LLL one year after surgery was 32%, increasing to 58% at eight years. Thus, 55% of

Table 4. — *Risk factor analysis by Cox regression*.

	Estimate	Hazard ratio (HR)	*p* value	95% CI for HR
Age	0.01	1.01	0.19	[0.99, 1.03]
BMI	-0.04	0.96	0.03	[0.92, 0.99]
Type of cancer				
Cervical	0.19	1.21	0.73	[0.40, 3.66]
Uterine	0.43	1.54	0.43	[0.52, 4.66]
Ovarian	-0.30	0.74	0.59	[0.25, 2.18]
Vulvar/Vaginal	1.11	3.04	0.33	[0.32, 28.48]
Site of lymphadenectomy				
Pelvic only	1.26	3.52	0.42	[0.16, 76.17]
Para-aortic	1.59	4.88	0.31	[0.23, 104.28]
Inguinofemoral	1.17	3.23	0.29	[0.38, 27.64]
No. of removed lymph nodes	0.02	1.02	0.01	[1.00, 1.03]
Positive lymph nodes	-0.04	0.96	0.86	[0.59, 1.56]
Radiotherapy	-0.43	0.65	0.12	[0.38, 1.11]
Chemotherapy	-0.01	0.99	0.99	[0.55, 1.78]
Postoperative infection	0.53	1.70	0.05	[0.99, 2.89]
Postoperative lymphocyst	0.70	2.01	0.0031	[1.27, 3.18]

Table 5. — *Risk factor selection by BIC*.

	Estimate	HR
No. of removed lymph nodes	0.018	1.018
Postoperative lymphocyst	0.724	2.062
Inguinofemoral lymphadenectomy	0.940	2.559

BIC: Bayesian Information Criterion; HR: Hazard Ratio.

documented LLL occurred within the first year after surgery. Median time to diagnosis of LLL was 5.2 years with a 95% confidence interval [3.00, infinite] (Figure 1).

Table 2 shows clinical characteristics and estimated prevalence of LLL one and eight years after surgery. Analysis of time to mild, moderate, and severe LLL revealed that the majority of affected patients suffered from mild or moderate edema. Only 8% and 11% developed severe LLL within one and eight years, respectively. Patients who underwent surgery for vulvar/vaginal cancer showed the highest probabilities of LLL when compared to other types of cancer. Furthermore, patients undergoing inguinofemoral lymphadenectomy developed LLL most frequently when compared to other sites of LN dissection. A total of 14.3% of all patients suffered from postoperative infection, developing LLL in 50% (73% after eight years) of these cases. In addition, patients with documented postoperative lymphocysts were at high-risk for developing LLL, 53% (75% after eight years) of them presented with LLL. Patient characteristics of the continuous variables are analyzed in Table 3. Median number of removed LNs was 27 (1-97), median BMI 24.7 (15.4-50.0), and median age 57 (24-89) years.

Potential risk factors associated with time-to-LLL were examined using Cox regression analysis (Table 4). The hazard for occurrence of LLL increased significantly by a hazard ratio (HR) of 1.02 with each additional LN removed ($p = 0.01$), CI [1.00, 1.03]. Postoperative local infection and lymphocysts significantly increased the hazard for development of LLL by a HR of 1.7 ($p = 0.05$) with a CI [0.99, 2.89] and a HR of 2.01 ($p = 0.0031$) with a CI [1.27, 3.18], respectively. Interestingly, the hazard for LLL decreased significantly by a factor of 0.96 ($p = 0.03$) for each additional BMI point with a CI [0.92, 0.99]. Patients with ovarian cancer, positive LNs, a past medical history of pre- and/or postoperative radiotherapy, or chemotherapy presented with a decreased risk to develop LLL when compared to patients without these characteristics. However, these differences were not statistically significant. Due to the small number of patients with dual primary cancers [9], this cohort was not suitable for a reasonable subgroup analysis.

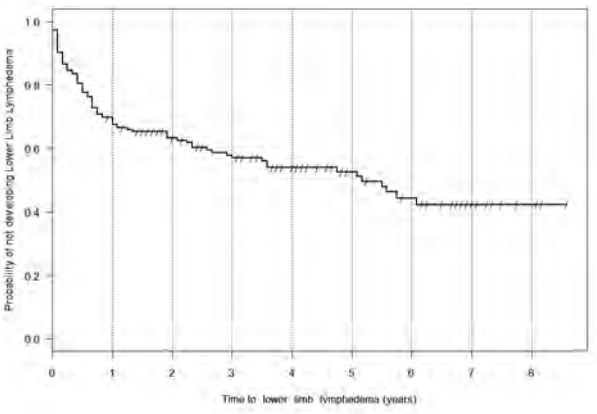

Figure 1. — Kaplan-Meier estimate of time to lower limb lymphedema.

The significant variables in the full model were confirmed by variable selection via BIC, revealing the risk factors with the strongest association to time-to-LLL (Table 5).

Discussion

This study estimated the prevalence of symptomatic LLL after lymphadenectomy in patients with newly-diagnosed gynecological malignancies and determined specific risk factors for its occurrence. Among 293 cancer patients undergoing surgical treatment, the authors report an estimated prevalence for LLL of 32% one year after surgery, increasing to 58% eight years after surgery. Thus, the present data clearly demonstrate that LLL is a frequent complication accounting for postoperative morbidity in these patients. Previous retrospective studies reported varying, but mostly lower prevalence of LLL [3, 4, 9-11]. Although patients had different follow-up times, it appears that no survival analytic methods were used to estimate the probability of LLL in these other studies. This lack of analysis, in addition to the absence of a standard definition for LLL, may account for some of the variability in prevalence rates for LLL reported. Furthermore, prevalence of LLL is strongly influenced by the employed detection method. Ryan *et al*. not only extracted data from medical records but also interviewed patients [4]. While 36% of the women reported swelling of their legs, diagnosis of LLL was made in only 18%, indicating that lymphedema is often overlooked if the patient does not complain of it. Two prospective studies, performing circumference measurements and/or volume displacement evaluations, reported incidences of LLL

higher than 40%, despite a shorter follow-up period [7, 13]. However, since patients in both studies self-reported swelling in only approximately 50% of detected cases, it remains unclear how specific these objective findings are. Future studies, implementing serial circumference measurements, and a follow-up period of several years, should reveal whether this method reliably detects early lymphedema or whether variations in leg circumferences are in some cases rather transitory postoperative findings. To date, standardized diagnostics are not available; however, tools such as circumference measurements, volume displacement evaluation methods, and the gynecologic cancer lymphedema questionnaire (GCLQ) [20] may be helpful for future studies.

In the present study, the majority of LLLs were diagnosed within the first year after surgery, which has also been reported by several other researchers [3, 11]. However, according to the Kaplan-Meier plot used in this study, the estimated median time to onset was 5.2 years. Lawenda and colleagues stated that awareness and assessment of signs and symptoms of LLL is essential immediately after surgery in order to initiate timely treatment, if necessary, and avoid chronicity and reduction in quality of life [5]. The results in the present study indicate that raising patient awareness is not only important immediately after surgery, but also during the years of oncological follow-up. Abu-Rustum *et al*. and Füller *et al*. previously documented a positive association between the number of dissected LNs and the prevalence of LLL [9, 10]. These results are consistent with the present findings showing a significantly increased risk for the development of LLL (HR 1.02) with each LN removed. The authors further substantiate their findings with the reduced model, confirming the association between the number of removed LNs and LLL. In this study, only 28% (82/293) of patients undergoing lymphadenectomy presented with a positive LN status post-surgery. Thus, 72% of patients did not directly benefit from lymphadenectomy; nevertheless, they had to face the high morbidity rates associated with this procedure. Further studies are urgently needed to evaluate the benefit of lymphadenectomy at different stages of gynecological malignancies and the value of sentinel node dissection in early cancer stages.

The present analysis clearly identified the postoperative formation of lymphocysts and occurrence of local infections as significant independent risk factors for the development of LLL. To the authors' knowledge, this is the first time that an association between lymphocysts and development of LLL has been shown. However, several authors reported lymphocyst formation as a common finding after pelvic lymphadenectomy, with most of them being asymptomatic [14, 21, 22]. Prospective studies are needed to evaluate whether patients with LLL following lymphadenectomy indeed show an increased incidence of lymphocysts and whether an early intervention is indicated to avoid development and persistence of LLL. Leminen *et al*. previously reported a significantly higher incidence of LLL in patients with wound infections after surgery for vulvar cancer [16]. The present study confirmed these findings, showing that postoperative infection is a significant risk factor for LLL in patients with gynecological malignancies. Whether infection is already a complication facilitated by impaired drainage remains to be investigated.

The reduced statistical model revealed that patients undergoing inguinofemoral lymphadenectomy are at a particularly high-risk for developing LLL, although the saphenous vein has been preserved in most of the patients. Similar to these results, it has been documented that inguinofemoral lymphadenectomy, as part of the standard surgical management for vulvar cancer, was frequently associated with postoperative chronic LLL [12, 23]. This may, at least in part, explain the highest prevalence of LLL in patients with vulvar/vaginal cancer also reported in previous studies [3, 4]. More recently, sentinel node dissection became part of the standard surgical treatment in early-stage vulvar cancer, reducing postoperative morbidity without compromising survival rates [24-26]. More studies are required to evaluate whether sentinel lymph node techniques are applicable to various gynecological cancers, which would greatly reduce postoperative morbidity including the prevalence of LLL.

Radiotherapy was not a risk factor for LLL in the present study. Similar results were reported by Gaarenstroom *et al*. [12]. However, previous studies investigated different gynecological malignancies separately and showed a significantly higher incidence of LLL after radiotherapy in patients with uterine cancer [11] and cervical cancer [3]. Werngren *et al*. reported a significantly higher ratio of patients with a history of combined external radio- and brachytherapy developing LLL compared to those exclusively receiving brachytherapy [7]. The present patient sample was more heterogeneous, including patients with various types of gynecological malignancies and receiving radiotherapy with dosages ranging from 20 Gy to 122 Gy. A more detailed analysis of different types and dosages of radiotherapy may provide further insights into its relevance as a risk factor for LLL and should be a subject of future studies. However, the subgroups in this study were too small to be suitable for subgroup analyses.

Interestingly, in the group of patients, a higher BMI had a protective effect for the development of LLL. Other investigators did not find an association between BMI and development of LLL [4, 9]. However, Kizer *et al*. observed in patients with cervical carcinoma that a lower BMI was associated with a significantly increased rate of postoperative complications, including LLL [27]. Furthermore, his group showed that the majority of patients lost weight during treatment. The present authors only documented patients' BMI at the beginning of treatment, which may somewhat limit the conclusions that can be drawn from this particular finding. Moreover, a positive association between BMI and incidence of lymphedema of the upper limb has been well-established in breast cancer patients following axillary lymphadenectomy [28, 29].

Conclusion

The aim of modern therapy of gynecological malignan-

cies is to minimize treatment-related morbidity, without compromising survival rates. Therefore, prevention of LLL through early detection and appropriate treatment, when necessary, has to be an important aim in the care of patients who undergo lymphadenectomy. In order to adequately inform patients and choose the most appropriate treatment option available, the clinician has to be aware of potential risk factors, such as postoperative infection or lymphocyst formation, and the impact of extended LN dissection, specifically within the inguinofemoral region. Until a significant reduction in LLL prevalence has been achieved through individualized therapy, patients should be educated about the risk of developing lymphedema, ways of prevention, symptoms, resources, and options for treatment. Although this is not the primary concern at the time of cancer diagnosis, it may become highly important after therapy.

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Invasive cancer of the cervix: does the UK National Health Service screening programme fail due to patients' non-attendance?

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Summary

The UK National Health Service (NHS) cervical screening programme aims to prevent invasive cancer of the cervix, yet this programme fails in some women. Women diagnosed with cancer of the cervix at a colposcopy unit in the North East of England between April 1, 1997 and December 31, 2004 had cervical cytology histories classified. Thirty-seven cases were identified (median age 37 years; range 22-72 years). At six months before diagnosis, 24.3% had never undergone cytology screening (38.4% Stage IB+, 12.5% Stage IA). In addition, 59.5% of all cases were under-screened (when using criteria that included screening was 'up to date' if less than five years had elapsed between last negative test result and their diagnosis). Women in this case series failed to attend regular cervical screening, with those never attending screening more likely to present with advanced cancer.

Key words: Invasive cancer of cervix; NHS cervical screening programme; Cervical cytology.

Introduction

In the UK the National Health Service (NHS) cervical screening programme aims to reduce the incidence of and mortality from invasive cancer of the cervix by offering regular, free cervical cytology sampling to women at risk. Identifying and treating those with cervical intraepithelial neoplasia has halved the incidence and mortality of invasive cancer of the cervix in England since 1988 when the screening programme was introduced [1]. Other European cervical screening programmes have also shown a similar success. A Swedish case-control study, for example, investigating nearly all the cases of invasive cancer of the cervix between 1991 and 2001 has shown that women who adhered to screening guidelines were far less likely to develop invasive cancer (relative odds 0.21 95% confidence interval 0.16-0.28) [2].

Unfortunately despite the screening programme being highly effective in preventing cancer of the cervix, there were still 2,276 women who presented with this invasive disease in 2007 and 830 women died as a result of this cancer in 2008 [1]. It was reported in 2005 that women who failed to attend cervical cytology screening make up a disproportionately high number of cases of cancer of the cervix [3]. The most recent NHS cervical screening programme audit includes a classification of cytology histories from 6,231 women with cancer of the cervix diagnosed between April 2007 and March 2010 and 18,783 controls. It found 25.3% with Stage IB+ cases and 19.3% with Stage IA cases had no previous cytology [1]. Women with fully-invasive (Stage 1B+) cervical cancer were also less likely to have been screened regularly for the last eight years than were women in general.

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The aim of this study was to analyse attendance for cervical screening in women prior to their diagnosis of cancer of the cervix in the North East. A second aim was to determine if those with a poor screening history presented with more advanced cancer. Non-attendance for screening was reported as an important factor in the London series, but would the same be true for a population that has the second highest incidence rate for cancer of the cervix in England (age-standardised incidence rate of 10.3 per 100,000 female population between 2004-2008)? [4].

Materials and Methods

The regional colposcopy unit database was searched for patients who had attended the service with a histological diagnosis of cancer of the cervix between April 1, 1997 and December 31, 2004. For each of these cases a search was made using the National Health Authority Information System (NHAIS) to identify any previous cervical cytology tests. This database keeps a record of the date and results of each individual test along with the advised actions.

Cervical screening was offered to all women in the North East of England aged 20-64 at five yearly intervals between 1988 and 1997. From March 1997 the interval was reduced to three yearly and in October 2003 the NHS Cervical Screening Programme in England raised the age of the first invitation letter to 25 years with a three-yearly interval up to age 49 and five years thereafter to 64 years. Consequently the cases in this series were invited at variable intervals depending on their age.

Any samples that were taken in the six months prior to diagnosis were excluded from the classification process to avoid including tests that were precipitated by the presence of symptoms or were performed as an additional diagnostic aid. For simplicity, women were classified as being 'up to date' if five years or less had elapsed between the date of the last negative screening sample resulting in routine recall, and the diagnosis. It is accepted that some women would have been invited three

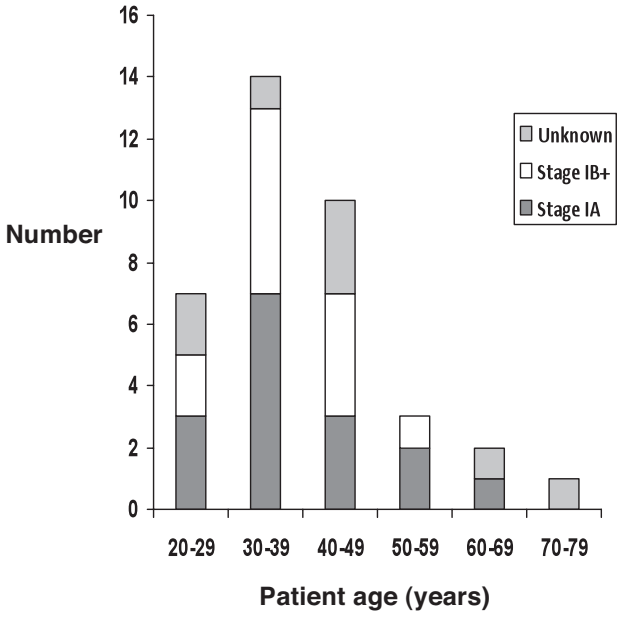

Figure 1. — Age at diagnosis of women with invasive cancer of the cervix.

yearly prior to a diagnosis and that five years is a generous allowance. For women on an early recall action code due to a previous inadequate, borderline, or mild cytology result, their screening was classified as being 'up to date' if one year and three months or less had elapsed between the date of the last screening sample and the diagnosis. For women over 65 years, their screening was 'up to date' if they had one negative test result between the ages 60 and 65 years. For women with a test indicating a need for further investigation and/or treatment at colposcopy, they were classified as being 'up to date' if they had at least one negative test result following the recommendation for colposcopy. The hospital's histology results database was searched to obtain International Federation of Gynaecology and Obstetrics (FIGO) tumour stage for the micro-invasive cancers and a minimum stage for the more advanced cases. The results were analysed for statistical significance using an exact contingency table test as described by Plackett [5].

Names, addresses, and unique identifiers such as NHS or hospital numbers were deleted and the personal identifier used was date of birth in order to anonymise the data. Research and ethics review was not required as the audit contained routinely collated data in an anonymised format.

Results

The case series included 37 women (median age 37, range 22-72). One woman had two diagnoses of invasive cancer separated by follow-up cytology samples within the time period of the study. She was included as a single case of Stage IB+. A woman aged 72 years was included as she was 61 years old when the screening programme commenced in 1988, and so would have been eligible for at least one screen before the age of 65.

Figure 1 shows the incidence of cervical cancer according to FIGO staging and age. Forty-three point three percent of cases were Stage IA, 34.2% were Stage IB+, and in 21.6% the final stage was not known. The most common histological diagnosis was squamous carcinoma reported in the majority of cases (87%). Three (8%) cases were adenocarcinoma and two (5%) were adenosquamous carcinoma.

Table 1. — *Cervical screening status at six months before diagnosis of invasive cancer of the cervix.*

Cervical screening history at six months before diagnosis	Stage IA n (%)	Stage IB+ n (%)	Unknown n (%)	Total n (%)
'Up to date'	8 (50)	2 (15.3)	5 (62.5)	15 (40.5)
Under-screened	8 (50)	11 (84.6)	3 (37.5)	22 (59.5)
Total	16 (100)	13 (100)	7 (100)	37 (100)

Table 1 shows the screening status of subjects six months or more before diagnosis - 40.5% of all cases were classified as being 'up to date' with their cervical screening examinations and 59.5% were lapsed or had never been screened. Just 15.3% of Stage IB+ cases were 'up to date' with their screening tests compared with 50% of those presenting with Stage IA cancer. The exact contingency table test for these gave a non-significant p value of 0.114.

Nine women (24.3% of all cases) had no cytology prior to the six-month exclusion period and, when divided according to FIGO Stage, 5/13 (38.4%) of Stage IB+ cases had no previous cytology compared with 2/17 (12.5%) of Stage IA cases (Figures 2 and 3). The p value of 0.19 using the exact contingency table test did not reach statistical significance. The ages at diagnosis of the women with no previous cytology were 30, 32, 37, 37, 37, 37, 40, 42, and 72 years. Of those presenting with Stage IA cancer, 43.8% had a negative sample within five years of diagnosis compared with 15.4% of Stage IB+ cases. Two women with Stage IB+ cancer were suspended from screening as they required diagnostic testing and possible treatment at colposcopy, but a negative sample was not obtained before the diagnosis was made.

Discussion

This case series confirms that women with invasive cancer of the cervix in the North East of the UK have a high rate of infrequent or non-attendance for cervical screening with 59.5% of all cases under-screened and 24.3% never screened six months or more before their diagnosis. It also shows that women with more advanced cancer are more likely never to have attended for cervical cytology (38.4% Stage IB+ cases compared with 12.5% Stage IA).

Brinkmann *et al*. published a series of 60 cases of invasive cancer of the cervix diagnosed in a London tertiary referral centre between 1988 and 1998 that coincided with the first ten years of the formal screening programme in England [3]. Fifty-five percent of these women were either non-attendees for screening or under-screened. The present local series began nine years after the introduction of the screening programme when women should have been more familiar with the screening programme and therefore more willing to participate. It is disappointing that these results show no improvement.

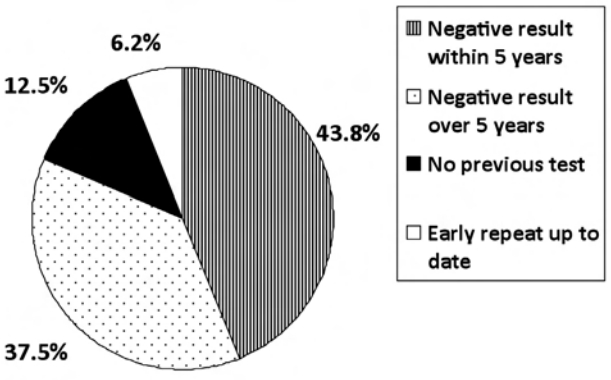

Figure 2. — Pie chart of cervical screening status at six months before diagnosis of Stage IA invasive cancer of the cervix.

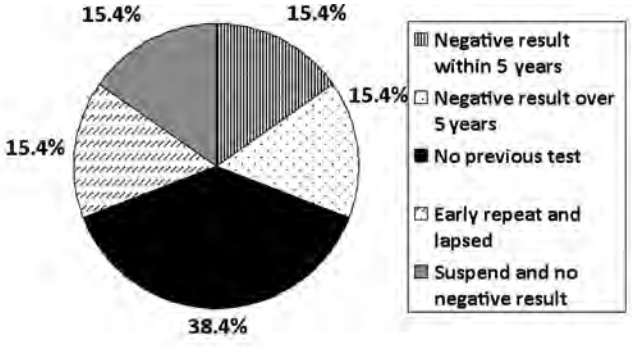

Figure 3. — Pie chart of cervical screening status at six months before diagnosis of Stage IB+ invasive cancer of the cervix.

The NHS cervical screening programme has provided a more detailed breakdown of the 2011 audit [1]. For the North East region, 23.3% of Stage IA cases and 30.8% of Stage IB+ cases were recorded as having no previous cytology, compared with 19.3% for Stage IA and 25.3% for Stage IB+ from pooled data for England. The attendance data for the recent nationwide audit (2007-2010) [1] and this local series (1997-2004) have similar methodology as both excluded cytology test results within six months of diagnosis. For the more advanced cases, the number of women with no previous cytology decreased from 38.4% to 30.8%, but for the women diagnosed with microinvasion, those without previous cytology increased from 12.5% to 23.3% between the two audit periods.

There are several weaknesses of this audit. The study would have been more informative if FIGO staging had been known for all cases. Many studies investigating cervical screening and cancer of the cervix [1, 6] include a control group of non-hysterectomised women who do not develop cancer. This type of analysis helps to identify different demographic or behavioural characteristics in those presenting with invasive cancer to age-matched women. This type of analysis was not possible for this series as cases were drawn from a specialist colposcopy unit and not primary care.

Human papilloma virus (HPV) self-sampling may be an alternative strategy for screening non-attendees of cervical screening programmes. Szarewski *et al*. found that HPV self-sampling increased participation among non-responders in a randomised trial comparing provision of self-sampling kits with a further invitation to attend for cytology testing [7]. Further studies are required to investigate the response to self-sampling in other parts of the country with different demographics to clarify whether this initiative will be successful throughout England.

Conclusion

A recent study confirmed that women with screen-detected cancers of the cervix have a far better prognosis than those presenting with symptoms [8]. This present audit also suggests that those with invasive cancers are infrequent or non-attendees of a screening programme. It is vital, therefore, that additional strategies are found to increase uptake of cervical screening across all age groups of eligible women to maximise the effectiveness of national screening programmes in preventing invasive cancer of cervix.

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Expression of E-cadherin in primary endometrial carcinomas: clinicopathological and immunohistochemical analysis of 30 cases

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Summary

Introduction: Decreased expression of E-cadherin has been associated with poorly differentiated endometrial carcinomas and poorer outcomes. *Aim:* The purpose of this study was to examine the distribution of E-cadherin immunohistochemical expression in specimens from primary endometrial carcinomas and its relation to classical clinicopathological prognostic factors. *Materials and Methods:* Surgically-resected tissues of 30 patients with primary endometrial carcinomas were studied. Histological type and grade, depth of myometrial invasion, lymph-vascular space invasion, fallopian tube or ovarian invasion, and the presence of tumoral necrosis were evaluated. Immunohistochemical examination was performed on deparaffinized four-μm-thick sections. *Results:* The mean age of patients was 65 years (± 11.41). The 63.54% of carcinomas were moderately/poorly differentiated. No statistical correlation was found between the score or intensity of E-cadherin immunohistochemical staining (strong or moderate positive expression) and the clinicopathological factors tested. *Conclusions:* The association of E-cadherin immunoreactivity with the standard clinicopathological factors seemed to be contradictory. The classical clinicopathological factors remain the most important prognostic parameters.

Key words: E-cadherin; Carcinoma; Endometrial; Endometrioid; Immunohistochemistry; Pathology.

Introduction

Cell-to-cell adhesion is mediated by cell surface glycoproteins known as cadherins, through a Ca^{2+}-dependent mechanism [1-3]. Cadherins are divided into subgroups on the basis of their tissue distribution: E-cadherin (epithelial), P-cadherin (placental), N-cadherin (neural), and L-cell adhesion molecule (liver) [3]. E-cadherin has five extracellular domains, an intracellular tail, and connects to the actin cytoskeleton through a complex with the cytoplasmatic catenin [4]. E-cadherin has been shown to play a central role in cellular organization and to mediate the transmission of extracellular signals to cells [1, 5]. The expression of E-cadherin is also critical for the regulation of apoptosis of tumor cells [1]. Reduction and loss of E-cadherin expression in cancer cells seems to destruct the junctional cell structure, affecting therefore intracellular adhesion and promoting tumoral progression and metastasis [6]. Absent or decreased expression of E-cadherin is more likely to be associated with poorly differentiated or non-endometrioid endometrial carcinomas and with poorer outcomes [2, 7, 8].

The purpose of the present study was to examine the distribution of E-cadherin immunohistochemical expression in formalin-fixed, paraffin-embedded tissue specimens from primary endometrial carcinoma tissues of Greek patients and its relation with classical clinicopathological prognostic factors such as histological type, histological grade, depth of myometrial invasion, and extent to the cervix.

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Materials and Methods

Patients

Surgically-resected tissues of 30 patients with primary endometrial carcinomas who underwent surgery between 2006 and 2009 were randomly selected. The following histopathological parameters were determined: histological type and grade, depth of myometrial invasion, lymph-vascular space, and fallopian tube or ovarian invasion, presence of tumoral necrosis, and extent to the cervix. Pelvic and para-aortic lymph nodes were not dissected in all patients. Endometrial carcinomas were graded according to the World Health Organization (WHO) System.

Histological analysis

For histological examination, endometrial carcinomas were routinely fixed with formalin, embedded in paraffin, thin-sectioned, and stained with hematoxylin and eosin (H&E). Four-μm-thick sections including sufficient quantities of neoplasm mass were mounted on silane-coated glass slides.

Immunohistochemical analysis

Antibodies used for labelling paraffin-embedded tissue sections were fixed in formalin. Pre-treatment of tissues with heat-induced epitope retrieval was performed for each antigen. Staining procedure was performed on deparaffinized sections using the standard avidin-biotin-peroxidase complex method with automated immunostainer and 45 minutes incubation at room temperature with the primary antibody. Antibodies of immunohistochemistry were anti-human syndecan-1 (anti-human CD138, clone M115, mouse monoclonal, 1:50 dilution, and anti-human E-cadherin (clone 4A2C7, mouse monoclonal, 1:100 dilution. The final stage involved the dehydration and coverage of tile.

Evaluation of immunohistochemistry

The score of immunohistochemical expression of E-cadherin was classified into the following four categories: 0: < 5% immunopositive cells; 1: 5%-25% immunopositive cells; 2: 25%-75% immunopositive cells; 3: more than 75% immunopositive cells. Staining intensity was defined as weak, moderate, and strong.

Statistical analysis

Categorical variables are presented as frequencies and percentages, and continuous parameters are shown in terms of means and its corresponding standard deviations. Pearson chi-squared test was used to evaluate the potential association between categorical variables. The Kruskal-Wallis test, a non-parametric analogue of analysis of variance, was applied to test the hypothesis that samples were from the same population. In case of two samples, the Wilcoxon rank-sum test (also known as Mann-Whitney two sample statistic) was used instead. The results were considered significant if the corresponding p value was < 0.05.

Results

The mean age of patients was 65 years (± 11.41). The sample included 25 (83.33%) endometrioid carcinomas and five (16.67%) clear cell and papillary serous endometrial carcinomas. Using the WHO system, the cases were distributed as follows: grade G1 (well-differentiated adenocarcinomas) 11 cases (36.67%); grade G2 (moderately differentiated adenocarcinomas) 14 cases (46.87%); and grade G3 (poorly differentiated adenocarcinomas) five cases (16.67%). The mean myometrial invasion was 0.57 (± 0.24). Lymph-vascular space and fallopian tube invasion was found in seven (23.33%) and four cases (13.33%) respectively, while ovarian invasion was not observed. Necrosis was detected in nine cases (30%). Eight patients showed 5%-25% immunopositive cells, other eight cases had 25%-75% immunopositive cells, and 14 patients showed more than 75% immunopositive cells. The age of patients was not significantly different between these three groups (p = 0.40).

Endometrioid carcinomas included five cases with 5%-25% immunopositive cells, eight cases with 25%-75% immunopositive cells, and 12 cases with more than 75% immunopositive cells. In the clear cell and papillary serous endometrial carcinomas groups, E-cadherin was immunohistochemically expressed in 5%-25% of cells in three cases, and in more than 75% of cells in two cases. The score of immunohistochemical expression of E-cadherin was not statistically different between the above-mentioned histological types (p = 0.13).

Among those histologically classified as G1, three cases showed 5%-25% immunopositive cells, two cases had 25%-75% immunopositive cells, and six cases showed more than 75% immunopositive cells. In histological grade G2, immunopositivity for E-cadherin was detected in 5%-25% of cells in three cases, in 25%-75% of cells in five cases, and in more than 75% of cells in six cases. Finally, in histological grade G3, two cases had 5%-25% immunopositive cells, one case showed 25%-75% immunopositive cells, and two cases had more than 75% immunopositive cells. No statistically significant association was detected between histological grades and scores of immunohistochemical E-cadherin expression (p = 0.82).

Among eight cases that showed positive immunostaining for E-cadherin in 5%-25% of cells, the myometrial invasion was 0.65 (± 0.27). In eight cases with 25%-75% immunopositive cells and in 14 cases with more than 75% immunopositive cells, myometrial invasion was 0.60 (± 0.30) and 0.50 (± 0.17) respectively. These differences were statistically insignificant (p = 0.42) (Figures 1 and 2).

In case of lymph-vascular space invasion, immunopositivity for E-cadherin was detected in 5%-25% of cells in two patients, in 25%-75% of cells in two cases, and in more than 75% of cells in three patients. In the absence of lymph-vascular space invasion, 5%-25% immunopositive cells were found in six cases, 25%-75% in six patients, and more than 75% in 11 cases. There was no statistically significant association between lymph-vascular space invasion and scores of immunohistochemical E-cadherin expression (p = 0.97).

In the presence of tumoral necrosis, immunohistochemical expression of E-cadherin was found in 5%-25% of cells in two cases, in 25%-75% of cells in two cases, and in more than 75% of cells in five cases. If necrosis was absent, the corresponding frequencies for the three categories of E-cadherin expression were six, six, and nine, respectively. As evidenced by the chi-squared test, tumoral necrosis was not associated with the immunohistochemical E-cadherin expression (p = 0.82).

Out of 30 cases, 20 exhibited intense expression of E-cadherin and ten showed expression of moderate degree. The mean age of patients was not statistically different between these two groups (p = 0.27).

Strong positive expression was observed in nine cases of histological grade G1, in eight cases of grade G2, and in three cases of histological grade G3. The corresponding frequencies for moderate expression were two, six, and two, respectively. No statistically significant association was observed between the intense of E-cadherin staining and the histological grade (p = 0.41).

Concerning endometrioid carcinomas, strong positive expression was observed in 18 cases and moderate in seven patients. In the group of clear cell and papillary serous endometrial carcinomas, the E-cadherin staining was strong in two cases and moderate in three cases. The histological type of tumor and intensity of E-cadherin staining were not statistically related (p = 0.17).

The mean myometrial invasion was 0.55 (± 0.22) in the 20 cases with strong positive expression of E-cadherin and 0.60 (± 0.27) in the remaining patients with moderate staining. This difference was statistically insignificant (p = 0.63).

The intensity of staining was strong in 15 cases without lymph-vascular space invasion and moderate in the remaining eight cases. On the other hand, strong expression of E-cadherin was observed in five cases with

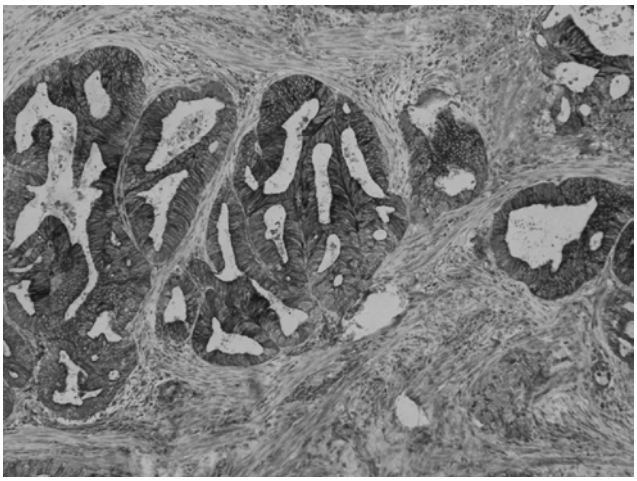

Figure 1. — Endometrial carcinoma: E-cadherin x 100.

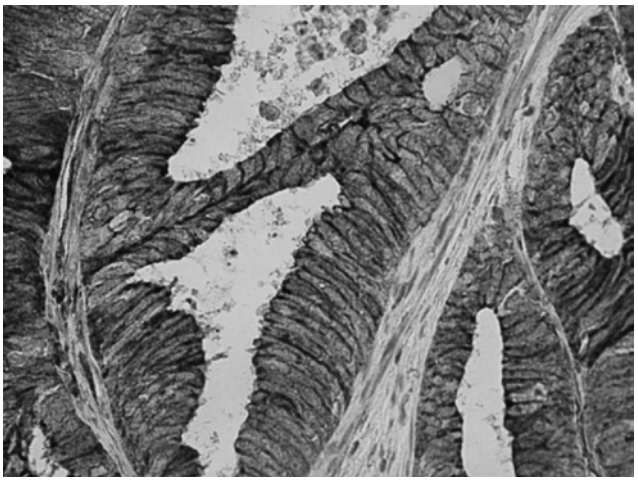

Figure 2. — Endometrial carcinoma: E-cadherin x 400.

lymph-vascular space invasion, while staining was moderate in the other two cases with tumor invasion in the lymph-vascular space. In statistical terms, the lymph-vascular space invasion and the intensity of E-cadherin staining were unrelated ($p = 0.76$).

In the presence of tumoral necrosis, the expression of E-cadherin was strong in five cases and moderate in four patients. Among cases without tumoral necrosis, 15 and six patients, respectively, showed strong and moderate E-cadherin staining. There was no statistically significant association between the presence of tumoral necrosis and the intensity of E-cadherin staining ($p = 0.40$).

Finally, in the presence of fallopian tube invasion, an equal number of cases (two) showed strong and moderate positive expression of E-cadherin. Once again, there was no statistically significant correlation between fallopian tube invasion and the intensity of E-cadherin staining ($p = 0.45$).

Discussion

Endometrial carcinoma is the most common malignancy of the female genital tract in developed countries with estimated 42,160 new cases diagnosed in the United States in 2009 [9]. In the majority of patients, the prognosis after primary therapy, typically total abdominal hysterectomy and bilateral salpingo-oophorectomy is excellent [10]. Patients with papillary serous or clear cell histology, tumor invasion depth to myometrium, poor tumor differentiation or extension of disease to other organs or lymph nodes within the pelvis, are at higher risk for disease recurrence [11]. However, better prognostic indicators are needed to identify which patients are most likely to develop extrapelvic metastases and thus, potential benefits from chemotherapy [8].

Cadherins represent an important class of the adhesion molecules, with an essential role in the homotypical cell-cell adhesion [12, 13]. The E-cadherin connects neighboring epithelial cells [12, 14]. The cellular adhesion depending on cadherins requires the formation of some compounds between E-cadherin and some cytoplasmatic proteins, known as catenins. The cytoplasmic part of E-cadherin interacts with the other components of adherens junctions, in particular the armadillo repeat proteins p120-catenin, γ-catenin/plakoglobin, and β-catenin. By binding of β-catenin to α-catenin the adherens junction complex is linked to the cortical actin cytoskeleton, thereby mediating mechanical stability. If the adherens junction components, in particular E-cadherin and β-catenin, are impaired, tumorigenesis is favored [12, 14-16]. E-cadherin has important roles not only in cell-cell adhesion, but also in tumor progression [6]. Decreased expression of E-cadherin is associated with a loss of cell-cell cohesive forces and has been shown to precede tumor cell motility [17]. Alterations of E-cadherin expression have been reported in many human tumors. In cholangiocarcinomas, E-cadherin expression was reduced in aggressive histological types [6, 18]. In the Rip-Tag mouse model of beta-cell tumors of the pancreas, E-cadherin loss was a prerequisite for progression from adenoma to invasive carcinomas, and in humans an inverse correlation between formation of entire adhesion complexes including E-cadherin/a-cetenin complexes, and survival of breast cancer patients has been demonstrated [19].

However, the expression of E-cadherin is the most important hallmark of epithelial differentiation. Its loss is a prerequisite for detachment, invasion, and finally dissemination and metastasis of neoplastic cells and can be linked directly to an activated Wnt signaling cascade, which triggers further malignant cell proliferation [20]. Loss of E-cadherin could be a trigger to induce expression of ZEB1, which was suggested to be important for maintenance of an invasive phenotype of epithelial cancer cells [21]. In primary colorectal carcinomas CHD1, gene repressors were found to be correlated significantly with the metastatic spread of the tumor [22]. Poor survival is

often the result of tumor's increased tendency for remote metastasis, as well-shown in non-endometrioid carcinomas which tend to a have an extra-uterine dissemination. The aggressive behavior of these carcinomas might be due to decreased intracellular cohesiveness in these tumors, as seen in the instance of E-cadherin loss [1].

Decreased E-cadherin expression has been associated with increased invasive and metastatic potential in endometrial carcinomas. The first report to show the relationship between E-cadherin expression and the grade of the tumor was Sakuragi *et al*. [5]. Myometrial invasion and lymph node metastasis were also taken into account. According to their findings, decreased E-cadherin expression in endometrial carcinoma was associated with deep myometrial invasion and higher grade of the tumor. E-cadherin expression patterns were also more frequently associated with para-aortic node metastasis than positive patterns. Their data suggested that decreased E-cadherin expression may be a risk factor for deep myometrial invasion, even when the histologic grade of the tumor is considered. However, in the event that E-cadherin is expressed, the possibility of a functionally abnormal molecule or local stimulants should be considered. Some other studies also showed a decreased E-cadherin expression in endometrial tumors. Scholten *et al*. investigated the expression of E-cadherin, alpha-catenin, and beta-catenin in endometrial carcinoma in order to determine the prognostic value of these factors [7]. Negative E-cadherin, alpha-catenin, and beta-catenin expression was observed in 44%, 47%, and 33%, respectively of endometrial carcinomas, and was correlated with histologic FIGO grade 3. Negative E-cadherin expression was more often observed in non-endometrioid endometrial carcinomas (NEECs) than in endometrioid carcinomas (75% vs 43%). Combined positive E-cadherin, alpha-catenin, and beta-catenin expression was an independent positive prognostic factor for survival in patients with grades 1 and 2 carcinomas, while negative E-cadherin expression was found to be associated with histologic grade 3 (7). Holcomb *et al*. found that tumor grade and histological type were identified as significant predictors of E-cadherin expression [3]. Nevertheless, when grade was controlled, endometrioid carcinoma remained 23 times more likely to express E-cadherin than papillary serous and clear cell carcinomas. According to the study of Singth *et al*., both human endometrial cancer specimens and cell lines, that when ZEB1 is inappropriately expressed in epithelial-derived tumor cells, E-cadherin expression is repressed, and that this inverse relationship correlates with increased migratory and invasive potential [23]. In another study, reduced E-cadherin immunoreactivity was seen in 44.8% of the endometrial carcinomas and 65.4% of the metastases with a statistical correlation to higher tumor grade only in metastatic lesions [24]. Mell *et al*. concluded that decreased E-cadherin expression is an independent prognostic factor for disease progression and mortality in pathological Stages I to III endometrial cancer and suggested that evaluation of E-cadherin expression may aid in the selection of patients for more aggressive adjuvant therapy [8]. In this study, the authors evaluated the immunohistochemical expression in formalin-fixed, paraffin-embedded tissue specimens from primary endometrial carcinomas tissue from Greek patients and clarified its role in relation to the standard clinicopathological factors. No statistical correlation was found between the score of intense of E-cadherin staining (strong or moderate positive expression) and histological grade and type, lymph-vascular space invasion (presence or absence), presence of tumoral necrosis, and when there was fallopian tube invasion. E-cadherin immunoreactivity did not associate with any of the standard clinicopathological factors tested. As already reported, experimental studies suggest that loss of E-cadherin expression in the tumor cells leads to cell detachment, as well as to malignant phenotype changes that are essential for the tumor cells to invade extracellular matrix [8]. E-cadherin may indeed be an invasion-inhibiting molecule. The question however, if E-cadherin in transformed cells is functionally normal, has not yet been answered and simply detecting E-cadherin may not be the appropriate way to assess tumor invasiveness.

Conclusions

More than 70% of E-cadherin expression was observed in endometrial carcinomas in Greek patients. The association of E-cadherin immunoreactivity with the standard clinicopathological factors seems to be contradictory. Therefore, at present, the classical clinicopathological factors remain the most important parameters for the evaluation of endometrial carcinoma prognosis.

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Cytology at the time of cervical colposcopy

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Summary

Objective: The efforts of the authors are to evaluate the role of performing a Papanicolaou (Pap) smear at the time of colposcopy. *Materials and Methods:* This retrospective chart review included patients from 2004 to 2009 who underwent cold knife cone (CKC) biopsy or loop electrosurgical excision procedure (LEEP) for cervical intraepithelial neoplasia types 2 and 3 (CIN 2 and 3) or patients with discrepancy between Pap and colposcopic results. All patients presented to the gynecology clinics in a tertiary care hospital. Results were compared which included: the abnormal Pap smear which led to referral for colposcopy, the Pap smear performed at the time of colposcopy, the colposcopic biopsy, and the excisional biopsy. Interpretation of results was calculated with Cohen's κ Statistics. *Results:* One hundred forty-seven patients qualified for the study. One hundred five patients had excisional biopsy proven high-grade squamous intraepithelial lesion (HSIL). Eighty-two of these high-grade excisional pathology results were preceded by high-grade Pap cytology at the time of colposcopy; however 23 Pap cytology results indicated either low-grade squamous intraepithelial lesion (LSIL) or negative (20 and 3 respectively), but were followed by an excisional procedure revealing high-grade pathology. Eighty-one colposcopic biopsies confirmed high-grade excisional biopsy pathology. However, 24 colposcopic biopsies were low-grade or negative (13 and 11 respectively), but followed by a high-grade excisional biopsy. *Conclusion:* The addition of a Pap smear at the time of colposcopy has the potential role of recognizing high-grade cervical dysplasia.

Key words: Colposcopy; Papanicolaou (Pap) smear; High-grade squamous; Intraepithelial lesion (HSIL).

Introduction

Since the 1950s, the steady decline in cervical cancer incidence and mortality has been attributed to widespread cervical cancer screening [1, 2]. The Papanicolaou (Pap) smear is the most commonly utilized method to detect these lesions; however occasionally results are reported as normal when abnormal cells are present (false negative). Additionally, results are sometimes reported as abnormal when there are only normal cells (false positive). Over 90% of Pap smears will be diagnosed "negative for intraepithelial lesion or malignancy" [3].

Other cytology results from Pap smears are stratified by severity, and can include: low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL), amongst others. Histology results are likewise classified as CIN 1, 2, and 3. CIN 1 is the most benign and considered a manifestation of infection with human papillomavirus (HPV). CIN 2 and 3 are progressively more severe precursors of cervical cancer [4]. HSIL cytology is consistent with a diagnosis of CIN 2 or 3 and can include: hyperchromatic nuclei, abnormal chromatin distribution, nuclear atypia or pleomorphism, and increased nuclear/ cytoplasmic ratio. Milder manifestations of these changes achieve a diagnosis of LSIL cytology or CIN I histology [5].

A large proportion of LSIL (61%) is destined to spontaneously regress in 12 months, especially in adolescents [6]. However, CIN 2 or 3 has a regression of only 35% [7]. HSIL patients are almost always managed with colposcopy and biopsy as a first step. However, colposcopy following HSIL cytology can miss a significant number of CIN 2 and 3 lesions. Therefore most women with HSIL eventually undergo an excisional procedure [8]. This procedure includes either the loop electrosurgical excision procedure (LEEP) or cold knife conization (CKC). LEEP consists of removing the transformation zone or squamocolumnar junction and can be performed under local anesthesia in an office setting [9]. CKC can be utilized to obtain endocervical tissue and it is achieved with a scalpel incision of the cervix under general anesthesia.

It is important to have an accurate diagnosis of cervical dysplasia as excisional procedures have associated risks greater than Pap and colposcopy. All excisional procedures including LEEP and CKC may predispose to low birth weight and preterm birth [10-12]. Whether this is truly causal and not simply an association remains unclear [13]. For example, risks of cervical dysplasia and preterm delivery each include smoking, low socio-economic status, and infection [14]. Jakobsson *et al*. found that surgery itself and not the background characteristics explain the increased risk for preterm birth [14]. Therefore, there is a need for an intermediate tool in the management of cervical dysplasia to increase the sensitivity of the recognizing high-grade dysplasia.

In this study, the authors' objective was to examine the value of Pap smear at the time of colposcopy as noninvasive means to improve the accuracy of the diagnosis of cervical dysplasia. This retrospective chart review compares both Pap at the time of colposcopy and the colposcopic exam alone to the final pathology of the excisional procedure.

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Materials and Methods

A retrospective chart review was performed and included patients undergoing LEEP or CKC for CIN 2 and 3 or discrepancy between Pap and colposcopy from January 1st, 2004 to December 31st, 2009.

Data was obtained from medical charts (pathology reports) and included the results of the last (most recent) abnormal Pap smear which led to referral for a colposcopic exam, results of the Pap smear performed at the time of colposcopy, colposcopic biopsy results, and the results of the excisional biopsy.

Women who had colposcopy performed followed by successive cone biopsy or LEEP were included in this study. Colposcopy was performed on patients with HSIL on Pap smear cytology, persistent LSIL, atypical squamous cells (ASC), or atypical glandular cells (AGC). Patients with cervical cancer were excluded due to the small number of cases to avoid statistical inconvenience, and patients with inconclusive biopsy results unable to be classified were also excluded. All colposcopic exams were performed and documented the same way; the key concepts of the system included: aceto-white reaction, color, margins, and vessels examined to formulate a colposcopic impression and to aid in the selection of the most appropriate sites for colposcopically-directed biopsy. Three percent acetic acid solution was applied to the surface to improve visualization of abnormal areas. Monsel's solution was applied with large cotton swabs to the surface of the cervix to achieve hemostasis.

Liquid-based technology Thin Prep, was utilized for all Pap smears in the present study. Cervical cells were transferred to the specimen vial with a broom-like device and immersed into liquid fixative, fixing the cells instantly.

In this study, the guidelines implemented were in accordance with the 2001 American Society for Colposcopy and Cervical Pathology and updated 2001 Bethesda classification.

The summaries on the measurement results (HSIL, LSIL, and normal) are displayed in frequency tables and Kappa coefficients are estimated to assess agreement between two measurement methods as any pair of the three methods: excisional biopsy, colposcopic biopsy, and Pap smear performed at the time of colposcopy. For most purposes, Kappa coefficients greater than 0.75 may be taken to represent excellent agreement, below 0.40 may be interpreted as poor agreement, and between 0.40 and 0.75 may be understood as intermediate agreement recommended by Landis and Koch [15].

Results

Institutional review board approval was obtained to review medical records for 147 patients who underwent an excisional procedure for cervical dysplasia. Of this total, 105 patients revealed high-grade pathology. Among these 105 patients, 82 cases were further diagnosed with Pap smear at the time of colposcopy and colposcopy as compared to 81 cases of high-grade dysplasia by colposcopic biopsy (Tables 1 and 2). Twenty-three Pap smears at the time of colposcopy were read as low-grade dysplasia or negative for dysplasia and were followed by a high-grade excisional biopsy (LSIL 20 cases and negative in three cases). Similarly 24 (LSIL 13 cases and negative in 11 cases) colposcopic biopsies were low-grade or negative for dysplasia, but preceded an excisional biopsy of high-grade dysplasia.

One hundred seven Pap results were HSIL. Of this group, six were low-grade and 19 were negative for dysplasia from the excisional pathology report. Ninety-nine colposcopic biopsies were high-grade dysplasia. Of this group, five were low-grade and 13 were negative for dysplasia from the excisional pathology evaluation.

The Pap smear and colposcopic biopsy together compared to excisional biopsy pathology is presented in Table 3.

Table 1. — *Cervical cytology at the time of colposcopy compared to subsequent excisional biopsy pathology*.

Pap smear at colposcopy	Excision biopsy HSIL	LSIL	Negative	Total
HSIL	82	6	19	107
LSIL	20	5	11	36
Negative	3	1	0	4
Total	105	12	30	147

HSIL = high-grade squamous intraepithelial lesion (CIN2-CIN3).
LSIL = low-grade squamous intraepithelial lesion (CIN1-HPV changes).

Table 2. — *Colposcopic biopsy compared to subsequent excisional biopsy pathology*.

Colposcopy	Excision biopsy HSIL	LSIL	Negative	Total
HSIL	81	5	13	99
LSIL	13	5	9	27
Negative	11	2	8	21
Total	105	12	30	147

Table 3. — *Pap smear and colposcopy together compared to excisional biopsy pathology*.

PAP/Colposcopy	Excision biopsy HSIL	LSIL	Total
H/H	63	12	75
	84.00	16.00	
	60.00	28.57	
H/L	19	13	32
	59.38	40.63	
	18.10	30.95	
L/H	18	6	24
	75.00	25.00	
	17.14	14.29	
L/L	5	11	16
	31.25	68.75	
	4.76	26.19	
Total	105	42	147

H = HSIL; L = LSIL.

Discussion

In the ASCUS-LSIL Triage Study (ALTS), the sensitivity of initial colposcopy for CIN3 identified during two years of observation was only 54% [16].

In the present study, Pap at the time of colposcopy and colposcopic exam had similar ratios (82 vs 81 cases) of making the diagnosis of high-grade dysplasia. Among the cases with high-grade excisional pathology, there were only three patients with false-negative results (2.8%). However this number was 11 cases (10.4%) for colposcopy. Therefore, Pap smear may have a lower false-

negative rate in comparison to colposcopy in the recognition of high-grade cervical dysplasia.

The two approaches: Pap at the time of colposcopy and colposcopy alone agreed on high-grade diagnosis for only 63 patients. Individually, 18 high-grade excisional biopsies were previously correctly diagnosed as high-grade by colposcopy with an incorrect "benign" Pap smear report. Nineteen high-grade excisional biopsies correlated with high-grade Pap reports with an incorrect low-grade colposcopic diagnosis. When these two techniques are utilized together, a total of 100 patients (95.2%) would be accurately diagnosed with high-grade cervical dysplasia.

Literature reveals the positive predictive value (PPV) of agreement of high-grade colposcopic impression with high-grade histology ranges from 39% to 70% [17, 18]. The false-positive rate of Pap smear and colposcopy to incorrectly diagnose high-grade dysplasia instead of low-grade or negative dysplasia in this study was 23.3% and 18.1%, respectively. Certainly inter-observer variability or spontaneous regression of the lesion may play a role. Possibly, the decrease in false-positives from colposcopy can be attributed to complete treatment of a small cervical lesion during the colposcopic biopsy.

In practice, the present clinicians perform a Pap smear at the time of colposcopy for patients that are referred to the Institution because the previous Pap is usually obtained from another institution and reviewed by pathologists outside this Institution. Additionally, there is an approximate delay of 88 to 124 days between Pap smear at the referring clinic and colposcopy at this Institution. The authors' efforts are focused on catching the possibility of spontaneous regression during this time and making the accurate diagnosis to increase the quality of care for the patients.

For physicians who do not routinely perform a Pap smear at the time of colposcopy, the additional Pap smear will increase care costs slightly. However, concurrent use of colposcopy and Pap smear increases the accurate diagnosis of high-grade cervical dysplasia, which may otherwise be missed by following the current guideline algorithms.

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Stage IB1 cervical cancer patients with an MRI-measured tumor size ≤ 2 cm might be candidates for less-radical surgery

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Summary

Objectives: To examine the correlation between histopathology and magnetic resonance imaging (MRI) measured tumor size and define whether patients with Stage IB1 cervical cancer with an MRI-measured tumor size ≤ 2 cm can be candidates for less-radical surgery. *Materials and Methods:* The authors retrospectively reviewed 200 patients with Stage IB1 cervical cancer who underwent radical hysterectomy (class III) and pelvic lymphadenectomy. The largest diameter of the tumor was determined by MRI in 52 consecutive cases. *Results:* Regarding risk factors for parametrial involvement, only tumor size and age are known before definitive surgery without conization. Multivariate analysis of these risk factors revealed that both tumor size and old age were independently associated with parametrial involvement. Eighty-eight patients had a tumor size ≤ 2 cm and an age ≤ 50 years, two of which (2.3%) had parametrial involvement. In 52 consecutive patients, a significant correlation between histopathology- and MRI-measured tumor size was found (r = 0.787). Twenty-three patients had an MRI-measured tumor size ≤ 2 cm, none of which had parametrial involvement. *Conclusions:* Patients with Stage IB1 cervical cancer lesions with a tumor size ≤ 2 cm measured by MRI and age ≤ 50 years can be treated with less-radical surgery.

Key words: Cervical cancer; Less-radical surgery; MRI.

Introduction

Patients with Stage IB1 cervical cancer are commonly treated worldwide with radical hysterectomy and pelvic lymphadenectomy. The most frequent site of the local spread of cervical cancer is the parametrium; parametrial spread occurs via direct microscopic extension or lymphatic channels. Therefore, the removal of the parametrial tissue is considered to be of paramount importance in the treatment of cervical cancer. However, parametrectomy is the main cause of postoperative complications, including bladder dysfunction, sexual dissatisfaction, and anorectal mobility disorders, which are attributable to partial denervation of the autonomic nerve supply to the pelvic organs during parametrial resection [1, 2]. Recent studies question the efficacy and safety of radical hysterectomy due to the high rate of long-term postoperative complications [3, 4]. Although nerve-sparing surgery may minimize these complications, no prospective randomized controlled trial to evaluate this surgery has yet been conducted.

Stage IB1 cervical cancers are defined by a broad range of tumor characteristics such as tumor size, depth of invasion, lymph vascular space invasion (LVSI), lymph node metastasis, and parametrial invasion. Recently, the authors reported that patients with a tumor depth of invasion ≤ 10 mm (or tumor size ≤ 2 cm), no LVSI, and age ≤ 50 years could be considered for less-radical surgery, such as modified radical hysterectomy or simple hysterectomy with pelvic lymphadenectomy [5]. Although the depth of invasion and LVSI can be assessed by a pathological examination of the cone biopsy specimen, tumor size may be determined by magnetic resonance imaging (MRI) before definitive surgery.

The objective of this study was to examine the correlation between tumor sizes measured by histopathology and MRI. In addition, the authors aimed to define whether patients with an MRI-measured tumor size ≤ 2 cm can be candidates for less-radical surgery.

Materials and Methods

The study population consisted of 200 patients who presented with Stage IB1 cervical cancer according to the 1995 International Federation of Gynecology and Obstetrics (FIGO) staging system treated with radical hysterectomy (type III) and systematic pelvic lymphadenectomy at the Okayama University Hospital, Japan, between 1985 and 2009. All hysterectomy specimens were collected and processed in a routine manner. The pathological factors evaluated included histology, depth of invasion, tumor size, LVSI, parametrial invasion, pelvic lymph node metastasis, and ovarian metastasis. The entire parametrial tissues of all specimens were submitted for microscopic examination. Parametrial involvement was classified as direct microscopic extension, metastasis of parametrial lymph nodes, or lymph vascular spread. The details are described previously [5]. MRI was performed on 1.5-T superconducting system. T2-weighted turbo spin echo images were acquired with a TR of 3,000 ms and a TE of 98 ms in the axial and sagittal planes of the uterine cervix. The matrix size was 512 × 256 pixels, and the section thickness was six mm. In 52 consecutive patients, the largest diameter of the tumor was determined from T2-weighted images.

Univariate analysis was performed using the chi-squared test and Spearman's rank correlation test. Multivariate analysis was performed using stepwise logistic regression analysis. Statistical significance was set at $p < 0.05$.

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Results

Patient characteristics are shown in Table 1. Overall, 20 (10.0%) of the 200 patients exhibited parametrial spreading. The authors previously showed that old age, depth of invasion, tumor size, LVSI, positive pelvic nodes, and ovarian metastasis are statistically associated with parametrial involvement in this patient population, whereas histologic subtype is not [5]. Among these risk factors, the authors can only know the tumor size and age before definitive surgery without conization. Multivariate analysis of these risk factors revealed that both tumor size (HR: 1.11, 95% CI: 1.04-1.19, $p = 0.003$) and old age (HR: 1.05, 95% CI: 1.01-1.10, $p = 0.022$) were independently associated with parametrial involvement. One hundred nineteen patients had a tumor size ≤ 2 cm, five of which (4.2%) had parametrial involvement (Table 2). In patients with a tumor size ≤ 2 cm, the frequency of deep stromal invasion was significantly higher in patients over 50 years old (Table 3). Eighty-eight patients had a tumor size ≤ 2 cm and were younger than 50 years, two of which (2.3%) had parametrial involvement (cases 1 and 3 in Table 3). Case 1 exhibited direct invasion in the parametrium as well as ovarian metastasis. Case 3 exhibited lymph vascular spread in the parametrium.

In 52 consecutive patients, the largest tumor diameter was determined by MRI. The median MRI-measured tumor size was 22 mm (range, 0-35). In comparison, the median histopathology-measured tumor size was 22.5 mm (range, 9-34). There was a significant correlation between histopathology and MRI-measured tumor sizes ($r = 0.787$, $p < 0.0001$) (Figure 1). Twenty-three patients had an MRI-measured tumor size ≤ 2 cm, none of which had parametrial involvement and three (13.0%) had positive pelvic lymph nodes (Table 4). Twenty-nine patients had an MRI-measured tumor size > 2 cm, four of which (13.8%) had parametrial involvement and seven (24.1%) had positive pelvic lymph nodes (Table 4).

Table 1. — *Patient characteristcs*.

Variables	No. of patients (%) (n = 200)	Variables	No. of patients (%) (n = 200)
Age (years)		Parametrial invasion	
Median	42	Negative	180 (90%)
Range	25-71	Positive	20 (10%)
Histology		Lymph node metastasis	
SCC	121 (61%)	Negative	171 (86%)
AD	56 (28%)	Positive	29 (15%)
ADSQ	23 (12%)		
Depth of invasion (mm)		Ovarian metastasis	
Median	9	Negative	198 (99%)
Range	2-25	Positive	2 (1%)
Tumor size (mm)		Adjuvant therapy	
Median	19	None	126 (63%)
Range	7-38	Radiotherapy	28 (14%)
LVSI		Chemotherapy	23 (12%)
Negative	111 (55%)	Chemoradiation	23 (12%)
Positive	89 (45%)		

SCC: squamous cell carcinoma; AD: adenocarcinoma; ADSQ: adenosquamous cell carcinoma; LVSI: lymph vascular space invasion.

Table 2. — *Patients with parametrial involvement with a tumor size of 2 cm or less*.

Case	Age	Histology	DOI (mm)	TS (mm)	SI	LVSI	LNM	OM	Type of PI
1	38	AD	6.5	14	≤ 2/3	+	–	+	direct
2	66	SCC	11	20	> 2/3	+	–	–	direct
3	48	SCC	5	10	≤ 2/3	+	–	–	LVSI
4	63	SCC	8.9	18	> 2/3	–	+	–	direct
5	51	SCC	9	16	> 2/3	+	+	–	direct

DOI: depth of invasion; TS: tumor size; SI: stromal invasion; LVSI: lymph vascular space invasion; LNM: lymph node metastasis; OM: ovarian metastasis; PI: parametrial involvement; AD: adenocarcinoma; SCC: squamous cell carcinoma.

Table 3. — *Relationship between age and deep stromal invasion in patients with a tumor size of 2 cm or less*.

Age (years)	Stromal invasion > 2/3	*p* value
150 or under	5/87 (5.7%)	0.0002
Over 50	10/32 (31.3%)	

Table 4. — *Relationship between MRI-measured tumor size and the presence of metastatic disease to the parametrium or pelvic lymph nodes*.

MRI-measured tumor size	Parametrial involvement	Nodes positive
Two cm or less (n = 23)	0 (0.0%)	3 (13.0%)
More than two cm (n = 29)	4 (13.8%)	7 (24.1%)

Discussion

Small IB1 lesions may be first documented by cervical conization before definitive surgical therapy. The authors previously reported that patients younger than 50 years with a tumor depth of invasion of ≤ 10 mm (or tumor size ≤ 2 cm) and no LVSI as assessed by cone biopsy can be considered for less-radical surgery, such as modified radical hysterectomy or simple hysterectomy with pelvic lymphadenectomy. However, evident IB1 lesions documented by clinical examination or MRI are generally treated by radical hysterectomy and pelvic lymph adenectomy without conization; therefore, additional studies are needed to elucidate whether some patients with IB1 lesions without conization can be treated with less-radical surgery.

The authors found that 119 patients had a tumor size ≤ 2 cm, five of which (4.2%) had parametrial involvement. The incidence of parametrial involvement of 4.2% may not be acceptable. Multivariate analysis showed both tumor size and old age to be independent predictors of parametrial spread; both factors also seem to be important risk factors for parametrial involvement. The authors demonstrated that deep stromal invasion is not uncommon in elderly patients, even if the tumor size is ≤ 2 cm; this is why old age is an important factor for parametrial involvement. Therefore, tumor size ≤ 2 cm and patients aged ≤ 50 years were selected as the low-risk subgroup criteria for parametrial spread and demonstrated that the risk for parametrial spread was 2.3% (2 / 88). One case exhibited direct invasion in the parametrium in addition to ovarian metastasis. As ovarian metastasis was suspected before operation upon MRI, less-radical surgery could not be performed. Other patients had only lymph

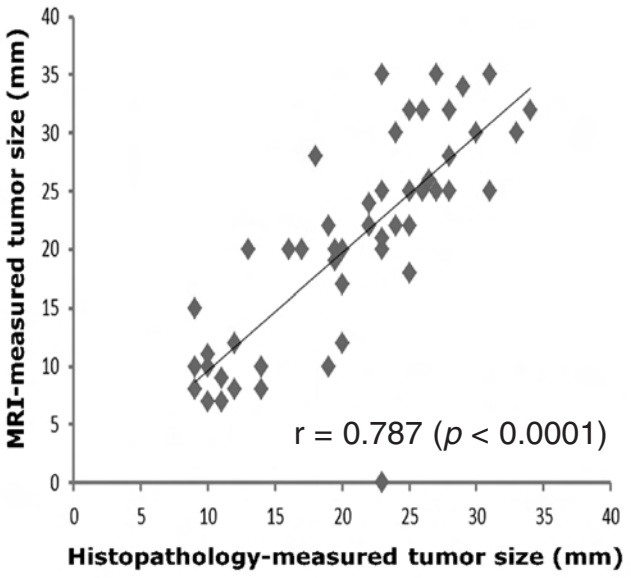

Figure 1. — Significant correlation between histopathology- and MRI-measured tumor sizes (r = 0.787, $p < 0.0001$).

vascular spread in the parametrium without evidence of pelvic lymph node metastasis. Frumovitz et al. state that it remains unclear what happens to these microscopic tumor emboli once they reach a draining lymph node-whether they implant and become a site of tumor metastasis or if the body's immune system clears this decidedly small-volume disease [6]. At present, the clinical significance of lymph vascular spread in the parametrium is undetermined. Therefore, it seems justifiable to assume that patients with a tumor size ≤ 2 cm and age ≤ 50 years do not need more radical parametrectomy.

Fertility-sparing vaginal radical trachelectomy (VRT) or abdominal radical trachelectomy (ART) in selected young women with Stage I cervical cancer has become an acceptable oncologic practice worldwide. ART is described as more radical than VRT with more parametrial tissue taken laterally during the procedure. In their review article, Rob et al. report that the oncological results of VRT and ART are similar for tumors ≤ 2 cm [7]. This may support the oncological safety for less-radical surgery in young patients with Stage IB1 lesions with a tumor size ≤ 2 cm.

Nowadays, MRI is considered the most accurate diagnostic tool to preoperatively detect tumor size [8-10]. The authors also observed a good correlation between the tumor size assessed by histological sections and MRI. In the present study, there was no parametrial involvement in the 24 patients with tumors ≤ 2 cm measured by MRI. This study however has some limitations including the possibility of over- or under-diagnosis. In fact, in a few patients, the size of the tumor was ≤ 2 cm when measured by MRI, but > 2 cm when measured by histopathology. Furthermore, there was a patient with a tumor size of more than two cm for whom MRI failed to detect any cervical tumor in accordance with previous studies [11, 12]. Kamimori *et al*. did not observe parametrial involvement in any of their 58 patients with a tumor size ≤ 2 cm measured preoperatively by MRI [12].

Conclusion

The current findings demonstrate that some patients with Stage IB1 cervical cancer lesions with a tumor size ≤ 2 cm measured by MRI and an age ≤ 50 years could be considered for less-radical surgery, such as modified radical hysterectomy and pelvic lymphadenectomy; however, further study is needed to clarify this issue.

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Polymorphisms of glutathione-s-transferase M1, T1, and P1 genes in endometrial carcinoma

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Summary

Objective: To investigate the polymorphism rates and possible roles of glutathione-s-transferase M1, T1, and P1 gene polymorphisms in the predisposition to endometrial cancer in Caucasian women. *Materials and Methods:* Serum samples and medical records were collected from 53 Caucasian women with newly diagnosed endometrial cancer and 67 women of the same race without any known history of cancer. Multiplex polymerase chain reaction (PCR) analysis was used to assess glutathione-s-transferase M1 (GSTM1) and T1 gene polymorphisms. Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method was used in salvage of GSTP1 gene polymorphism. *Results:* Frequencies of GSTM1 and GSTT1 null genotypes were not significantly different between the controls and patients with endometrial cancer (56.7% vs 52.8%, p = 0.671; 32.8% vs 26.4%, p = 0.574, respectively). The authors were not able to demonstrate any association between neither GSTP1 genotypes nor allele frequencies and endometrial carcinoma in the population studied (p = 0.310, p = 0.318, respectively). Moreover, no significant association could be demonstrated with GSTM1 and GSTT1 polymorphisms and clinical stages of endometrial cancer. Nevertheless, there was a significant difference between the frequencies of GSTP1 AA and GG genotypes in relation to Stage I disease when compared with advanced stages of endometrial carcinoma (p = 0.03). In addition, no association was found between polymorphisms of GST suspergene family and non-endometrioid type endometrial carcinomas. *Conclusion:* These results suggest that GSTT1, GSTM1, and GSTP1 polymorphisms are not associated with endometrial cancer in the Caucasian population.

Key words: Polymorphism; Gene; Glutathione-s-transferase; Adenocarcinoma; Carcinoma; Endometrium.

Introduction

Endometrial cancer is the most common type of uterine cancer and the most common gynecologic cancer worldwide [1, 2]. Prolonged unopposed estrogen stimulation, partial estrogen agonist drugs like Tamoxifen, late menopause, nulliparity, and obesity are the major known risk factors in the development of endometrial cancer [1, 3]. As well as the influence of environmental and individual etiological factors, genetic predisposition plays an important role in multifactorial process of carcinogenesis [4]. Common DNA polymorphisms in low penetrance genes, one of which is glutathione-s-transferase (GST) supergene family are addressed as one of the genetic factors to modulate an individual's susceptibility to malignancy [5-7].

The GST supergene family encodes dimeric enzymes which constitute a significant part of cellular enzymatic defense against exogenous chemicals and endogenous toxins that have carcinogenic potential [8]. The GST enzymes are involved in phase II detoxification reactions by conjugating a wide variety of suspected carcinogens, including aliphatic aromatic heterocyclic radicals, epoxides, arene oxides, and facilitate elimination of them [9]. Protection of the cells against oxidative stress by conjugation of reactive oxygen species with glutathione is the other vital function of GST enzymes [7, 8]. Therefore, normal or increased GST enzyme activity might be considered to protect somatic mutations in the DNA of susceptible tissues. Some researchers have asserted that GST enzymes are likely to modulate the induction of other enzymes in the conjugation process [10, 11]. In addition, it was shown that they could bind steroid hormones noncovalently and minimize the effects of short-term fluctuations in hormone levels in the extracellular environment [12, 13]. Consequently, they are considered to have a role in carcinogenesis of endometrial carcinoma which is a steroid hormone dependent cancer. Based on the structural, biochemical, and distributional characteristics, seven classes of GST enzymes covering alpha, mu, omega, pi, sigma, theta, and zeta subclasses are identified [14]. Among them, mu (coded from GSTM1), pi (coded from GSTP1), and theta (coded from GSTT1) enzymes are extensively studied because of their potential to modulate individual vulnerability to cancer.

Several GST loci exhibit genetic polymorphisms, many of which led to significant changes in GST enzyme activities. Polymorphisms of both subclasses GSTM1 and GSTT1 lead to alleles that are nonfunctional. Therefore, absence of enzyme activity is encountered with the presence of homozygous polymorphic alleles [15]. Similarly, 313A→G polymorphism on subclass GSTP1 resulting in Ile105Val substitution creates an allele that yields diminished GSTP1 enzyme activity [16]. There are ethnic variations in genotype frequencies of GST genes [6, 17-19]. Although the influences of GST genes were addressed in some cancers including lung, breast, and colorectal cancers so far [20-23], there have been very few reports on genotyping of these genes in endometrial cancer. The data concerning the role of polymorphisms of the GST genes in endometrial cancer were inconsistent [24-28].

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In this study, the authors investigated the relationship between the risk for development of endometrial cancer and polymorphisms of genes that play a role in detoxification reactions. Specifically, null polymorphisms on GSTM1 and GSTT1 genes and common GSTP1 Ile105Val mutation were studied in Caucasian patients with endometrial cancer and in controls. The possible additive effects of specific GST genotypes were also investigated.

Materials and Methods

Subjects

This study was conducted as a prospective case control study at the Department of Obstetrics and Gynecology, Uludağ University Medical School between November 2008 and October 2010. The study group consisted of 53 newly-diagnosed cases of endometrial cancer and 67 age-matched healthy volunteers with no cancer or chronic/devastating disorder history. Patients were recruited after histological diagnosis of endometrial carcinoma. Both groups visited the clinic during the same period. All the patients with endometrial cancer were primarily treated with surgical intervention. The clinical stages of endometrial cancer were staged and managed according to the recommendations of the International Federation of Gynecology and Obstetrics (FIGO) [29]. Demographic characteristics including age at diagnosis, height and weight were assessed in all women. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of the height (in meters). The patients and control subjects were from the same geographic region. Members of both the patient and control groups were asked to sign an informed consent form.

DNA extraction and GST genotyping

Blood samples of three ml from both the patient and the control groups were taken into ethylenediaminetetraacetic acid (EDTA) containing tubes. Genomic DNA was extracted from circulating leucocytes with salting out procedure by using DZ® DNA isolation kit (Dr. Zeydanlı Laboratories Ltd., Ankara, Turkey), and samples were stored in Tris EDTA buffer at -20°C until the time for polymerase chain reaction (PCR) analysis. All of the DNA samples collected from participants were studied and included in the present report without any elimination.

The GSTM1 and GSTT1 polymorphisms in the isolated DNAs were established by multiplex PCR method as described previously by Lin *et al*. [30]. For the GSTT1 polymorphism, forward 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and reverse 5'-TCA CCG GAT CAT GGC CAG CA-3' primers were used. To determine GSTM1 polymorphism, forward 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and reverse 5'-GTT GGG CTC AAA TAT ACG GTG G-3' primers were used. Albumin forward 5'-GCC CTC TGC TAA CAA GTC CTA C-3' and reverse 5'-GCC CTA AAA AGA AAA TCC CCA ATC-3' primers were used as internal controls. PCR was conducted by using 100 ng of genomic DNA, 500 pmol of each primer, 0.5 mM each of the dNTPs, one unit of Taq DNA polymerase, three mM $MgCl_2$, 50 mM KCl, ten mM Tris-HCl, 0.001% gelatin, pH 8.3 in a total volume of 25 µl. PCR conditions required denaturation for five min at 94ºC, followed by a second denaturation for 30 sec at 94ºC. Thirty-five cycles of amplification were conducted as follows: one min at 94ºC (denaturation), one min at 58ºC (annealing), one min at 72ºC (elongation), and finally ten min at 72ºC (final elongation). Genotypes were determined by migration of the products in agarose gel with added 2% ethidium bromide. GSTT1 459 bp, GSTM1 219 bp, and albumin 350 bp PCR products were produced.

Table 1. — *Demographic characteristics of the study population*.

	Patients (n = 53)	Controls (n = 67)	*p*
Age (years)¥	58 (35 - 83)	54 (44 - 75)	0.185
BMI (kg/m²)¥	29.94 (18.73 - 50.68)	29.13 (16.90 - 45.61)	0.226
Age of menarche (years)¥	12 (11 - 15)	13 (11 - 16)	0.057
DM			
Present	16 (30.2%)	11 (16.4%)	0.116
Absent	37 (69.8%)	56 (83.6%)	
HT			
Present	31 (58.5%)	33 (49.3%)	0.411
Absent	22 (41.5%)	34 (50.7%)	
Smoking habit			
Present	6 (11.3%)	5 (7.5%)	0.534
Absent	47 (88.7%)	62 (92.5%)	
Endometriosis			
Present	8 (%)	4 (%)	0.178
Absent	45 (%)	63 (%)	

¥ Values are given as a median (minimum-maximum); BMI: body mass index.

GSTP1 gene exon 5 Ile105Val polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described in previous work by Harries *et al*. [31]. For the GSTP1 polymorphism analysis, forward 5'-ACC CCA GGG CTC TAT GGG AA-3' and reverse 5'- TGA GGG CAC AAG AAG CCC CT -3' primers were used. PCR was conducted by using 50 ng of genomic DNA, 200 pmol of each primer, 0.3 mM dNTPs, one unit of Taq DNA polymerase, 1.5 mM $MgCl_2$, 50 mM KCl, ten mM Tris-HCl, 0.001% gelatin, pH 8.3 in a total volume of 25 µl. PCR conditions required denaturation for five min at 94ºC. Thermocycling which consisted of 35 cycles was conducted as follows: thirty sec at 94ºC (denaturation), 30 sec at 58ºC (annealing), one min at 72ºC (elongation) and finally ten min at 72ºC (final elongation). To identify the Ile105Val polymorphism, the amplification product was digested with 5U of Alw26 I enzyme (Genemark, Russia) in 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$ and one mM dithiothreitol and incubated at 55ºC for 16 hrs. Analysis conducted in 4% agarose gel after cutting with the enzyme. Genotypes were determined with the existence of distinct end products of 85, 91, and 176 bp. Interpretation of the results was as follows: if the 176 bp PCR product from the GSTP1 gene was cut into two distinct products of 85 bp and 91 bp, then the genotype was identified as Val/Val; if three distinct products formed as 176, 91, and 85 bp bands, then the genotype was identified as Ile/Val; and if the product was 176 bp then the genotype was identified as Ile/Ile.

Statistical analysis

Medians with minimum and maximum values were given for the descriptive variables of both groups. Statistics were performed using Statistical Package for the Social Sciences software version 17.0 (SPSS Inc., Chicago, IL, USA). Mann-Whitney U test was used for comparisons between the groups in terms of age, BMI, and age of menarche. In comparison of exposed risks and genotype frequencies, Pearson's chi-square test and Fisher's exact test were used, as appropriate. Hardy-Weinberg equilibrium was tested for allele and genotype frequencies of GSTP1 gene exon 5 Ile105Val polymorphism for the both groups. A p value of < 0.05 was accepted as a statistically significant difference.

Table 2. — *Association between GSTM1, GSTT1, GSTP1 genotypes and histological types, stages, and grades of endometrial carcinoma.*

		GSTM1		GSTT1		GSTP1			
	No. of patients	Active	*p*	Active	*p*	AA	AG	GG	*p*
Histological type									
Endometrioid adenocarcinoma	41	19 (46.0%)	0.965[§]	29 (70.7%)	0.480[§]	21 (51.2%)	18 (43.9%)	2 (4.9%)	1.00[◊]
Mucinous adenocarcinoma	6	1 (16.7%)		5 (83.3%)		3 (50.0%)	3 (50.0%)	0	0.225[†]
Clear cell adenocarcinoma	2	2 (100%)		1 (50.0%)		1 (50.0%)	0	1 (50%)	
Serous adenocarcinoma	1	0		1 (100%)		1 (100%)	0	0	
Mixed adenocarcinoma	3	2 (66.7%)		3 (100%)		0	2 (66.7%)	1 (33.3%)	
Stage									
I	39	19 (48.7%)	0.488*	28 (71.8%)	1.00*	20 (51.3%)	19 (48.7%)	0	1.00[#]
II	4	0	0.948[†]	3 (75.0%)	0.735[†]	2 (50.0%)	0	2 (50.0%)	0.501[¥]
III	6	4 (66.7%)		4 (66.7%)		2 (33.3%)	3 (50.0%)	1 (16.7%)	0.701[ø]
IV	4	2 (50.0%)		4 (100%)		3 (75.0%)	1 (25.0%)	0	0.03[ɣ]
Grade									
G1	19	10 (52.6%)	0.790[ɥ]	15 (78.9%)	NA[ɥ]	11 (57.9%)	8 (42.1%)	0	NA[ɥɥ]
G2	17	8 (47.0%)	0.758[ß]	11 (64.7%)	0.736[ß]	8 (47.0%)	8 (47.0%)	1 (6.0%)	NA[ßß]
G3	17	7 (41.2%)		13 (76.5%)		8 (47.0%)	7 (41.2%)	2 (11.8%)	
> ½ Myometrial invasion									
Present	24	10 (41.7%)	0.650	19 (79.2%)	0.599	13 (54.2%)	9 (37.5%)	2 (8.3%)	0.723[**]
Absent	29	15 (51.7%)		20 (68.9%)		14 (48.3%)	14 (48.3%)	1 (3.4%)	1[##]
Pelvic lymph node (LN) metastasis									
Present	5	3 (12%)	0.668	4 (10.2%)	1.00	2 (7.4%)	3 (13%)	0	0.651[**]
Absent	48	22 (88%)		35 (89.8%)		25 (92.6%)	20 (87%)	3 (100%)	1[##]
Para-aortic LN metastasis									
Present	4	2 (8%)	1.00	3 (7.7%)	1.00	1 (3.7%)	2 (8.7%)	1 (33%)	0.588[**]
Absent	49	23 (92%)		36 (92.3%)		26 (96.3%)	21 (91.3%)	2 (67%)	0.193[##]

NA, not applicable.
p value was calculated using Pearson chi square test with Yates correction to compare; [§]patients with endometrioid adenocarcinoma and non-endometrioid endometrial carcinomas; [◊]AA genotype and AG genotype frequencies in patients with endometrioid adenocarcinoma and non-endometrioid carcinoma of endometrium; [†]AA genotype and GG genotype frequencies in patients with endometrioid adenocarcinoma and non-endometrioid carcinoma of endometrium; *Stage I and II patients compared with Stage III and IV patients, [†]Stage I patients compared with advanced stages of disease; [#]AA genotype and AG genotype frequencies between patients in Stage I and II and patients in Stage III and IV; [¥]AA genotype and GG genotype frequencies between patients in Stage I-II and patients in Stage III-IV; [ø]AA genotype and AG genotype frequencies between patients in Stage I and patients in other stages; [ɣ]*p* value for analysis of AA genotype and GG genotype frequencies between patients in Stage I and patients in other stages; [ɥ]*p* value for the comparison of all grades of endometrial carcinoma, ɥɥin patients with AA genotype, and AG genotype; [ß]*p* value for the comparison of patients with G1 endometrial carcinoma and the other grades; [ɥß]in patients with AA genotype, and AG+GG genotype; **p value for the comparison of AA genotype with AG genotype; [##]*p* value for the comparison of AA genotype with AG+GG genotype.

Table 3. — *Association between GSTM1, GSTT1, and GSTP1 polymorphisms and endometrial carcinoma.*

	Patients n (%)	Controls n (%)	*p*
GSTM1			
active	25 (47.2%)	29 (43.3%)	0.671
null	28 (52.8%)	38 (56.7%)	
GSTT1			
active	39 (73.6%)	45 (67.2%)	0.574
null	14 (26.4%)	22 (32.8%)	
GSTP1			
AA	27 (50.9%)	28 (41.8%)	0.310
AG	23 (43.4%)	30 (44.8%)	
GG	3 (5.7%)	9 (13.4%)	
AA	27 (50.9%)	28 (41.8%)	0.318
AG + GG	26 (49.1%)	39 (58.2%)	
A allele	77 (72.6%)	86 (64.18%)	0.163
G allele	29 (27.3%)	48 (35.82%)	

Results

The demographic characteristics and exposure histories of the study population are shown in Table 1. The median age and median BMI between study and control groups did not differ significantly ($p = 0.185$ and $p = 0.226$, respectively). The authors analyzed 53 patients with endometrial adenocarcinoma and 67 healthy volunteers. Median age of menarche was insignificant between patients and control group (12 and 13-years-old, respectively; $p = 0.057$). The presence of diabetes mellitus (DM) and hypertension were not statistically significant between the patient and the control groups ($p = 0.116$ and $p = 0.411$, respectively). Similarly, smoking status and presence of endometriosis were not associated with the risk of developing endometrial adenocarcinoma ($p = 0.534$ and $p = 0.178$, respectively). Histological diagnosis of 41 patients was pure endometrioid carcinoma, while the histologic diagnoses of the remaining patients were as follows: six mucinous adenocarcinoma, two clear cell carcinoma, one high grade papillary serous carcinoma, and three mixed serous or clear cell type (Table 2). Of the patients with endometrial adenocarcinoma, 39 (73.6%) were in Stage I, four (7.5%) were in Stage II, six (11.4%) were in Stage III, and four (7.5%) were in Stage IV. Among the cases, 19 (35.8%) were in grade I, 17 (32.1%) were in grade II, and 17 (32.1%) were in grade III.

Table 4. — *Combined risk of GSTM1, GSTP1 genotypes with GSTT1 genotypes for developing endometrial carcinoma.*

	GSTT1 Active				GSTT1 Null			
	Patients	Controls	*p*	OR (95% CI)	Patients	Controls	*p*	OR (95% CI)
GSTM1 active, GSTP1 AA	6	6		1.0 (ref.)	5	4	1.000	1.25 (0.16 - 10.09)
GSTM1 active, GSTP1 AG/GG	10	12	0.916	0.83 (0.16 - 4.25)	4	7	0.680	0.57 (0.08 - 4.08)
GSTM1 null, GSTP1 AA	15	15	0.733	1.00 (0.22 - 4.64)	1	3	0.585	0.33 (0.01 - 5.99)
GSTM1 null, GSTP1 AG/GG	8	12	0.854	0.67 (0.12 - 3.54)	4	8	0.679	0.50 (0.07 - 3.45)

Table 5. — *Association between GSTM1, GSTT1 and GSTP1 genotypes and stages of endometrial carcinoma.*

	Stage I	Stage II	Stage III	Stage IV	$p^{\dagger}$	p*
GSTM1						
active	19	0	4	2	0.948	0.488
null	20	4	2	2		
GSTT1						
active	28	3	4	4	0.735	1.00
null	11	1	2	0		
GSTP1						
AA	20	2	2	3	1.0 (Ref)	1.0 (Ref)
AG	19	0	3	1	0.701[#]	1.00[#]
GG	0	2	1	0	0.03[$]	0.50[$]
GSTP1						
AA	20	2	2	3	0.685	0.627
AG + GG	19	2	4	4		

[†] Stages I was compared with Stages II+III+IV; *Stages I+II were compared with Stages III+IV.
[#] GSTP1 AA genotype was compared with AG genotype; [$]GSTP1 AA genotype was compared with GG genotype.

The distribution of GSTM1, GSTT1, and GSTP1 genotype frequencies in patients and controls are shown in Table 3. The GSTM1 active genotype was present in 25 (47.2%) cases and in 29 (43.3%) participants; the difference in their frequencies between both groups was not statistically significant (p = 0.671). Multivariate logistic regression model was applied for age, BMI, age of menarche, DM, hypertension, smoking, and endometriosis. Even after adjusting with the mentioned parameters, the authors did not find an association between presence of null GSTM1 genotype and endometrial adenocarcinoma (GSTM1 active versus null, OR = 1.514, 95% CI = 0.626-2.632). The GSTT1 active genotype was present in 39 (73.6%) and 45 (67.2%) of endometrial cancer patients and controls, respectively. When the groups were compared in terms of GSTT1 genotype frequencies, no statistical significance was determined (p = 0.574). Even after adjusting for age, BMI, age of menarche, DM, hypertension, smoking, and endometriosis in multivariate logistic regression model, presence of null GSTT1 genotype did not influence endometrial adenocarcinoma risk (GSTT1 active versus null, OR = 1.283, 95% CI = 0.735-3.197). As shown in Table 3, for the GSTP1 Ile105Val polymorphism, AA, AG, and GG genotype frequencies in endometrial cancer group were 27 (50.9%), 23 (43.4%), and three (5.7%), and in the control group were 28 (41.8%), 30 (44.8%), and nine (13.4%), respectively. There was no difference in frequencies of GSTP1 genotypes among the cases and the control group (p = 0.310). Furthermore, combined GSTP1 AG and GG genotypes were not associated with increased risk of developing endometrial adenocarcinoma when compared to AA genotype (p = 0.318). Even after adjusted age, BMI, age of menarche, DM, hypertension, smoking, and endometriosis, bearing AG or GG genotype, did not increase the risk of developing endometrial adenocarcinoma when compared to AA genotype (AG/GG vs AA, OR = 0.832, 95% CI = 0.548-1.975). GSTP1 A allele was present in 77 (72.6%) of the cases and 86 (64.18%) of the controls, whereas G allele was present in 29 (27.3%) and 48 (35.82%) of the cases and controls, respectively. Nevertheless, the difference in allele frequencies of GSTP1 gene did not reach statistical significance (p = 0.163).

The possible additive roles of combined GSTM1, T1, and P1 genotypes on the risk of developing endometrial carcinoma were calculated. Frequencies of cases and control individuals who were mapped according to the specific GST genotype display are shown in Table 4. The category including GSTM1, GSTT1, and GSTP1 AA genotypes was taken as the reference category. Nevertheless, the authors could not establish any significant association between any category and increased endometrial carcinoma risk.

Patients with endometrial carcinoma were also analyzed according to the clinicopathological characteristics of the cancer. Genotype statuses of the patients are shown with the stages of disease in Table 5. The differences in frequencies of GSTM1 and GSTT1 genes were not statistically significant in patients with Stage I disease when compared to patients in other stages (Stages II-IV) (p = 0.948, p = 0.735, respectively). Similarly, there was no difference in frequencies of GSTM1 and GSTT1 genes between patients with Stages I + II and patients with Stages III + IV (p = 0.488, p = 1.00, respectively). There was no significant difference in frequency of GSTP1 genotypes between Stage I and Stages II-IV, and Stages I + II and Stages III + IV, except for the frequency of GSTP1 GG genotype which was significantly higher among patients in advanced stage (Stages II-IV) disease than patients with Stage I disease when compared to AA genotype (p = 0.03).

Relationship between the frequencies of GSTM1, T1, and P1 genotypes and histological types, clinical stages and grades together with pelvic and para-aortic lymph node metastases in endometrial cancer are shown in Table 2. Frequencies of GSTM1, T1, and P1 genotypes between endometrioid and non-endometrioid histological types

were insignificant. Similar to the clinical stages of cancer, there was no significant difference between grade of endometrial carcinoma and frequency of all GST genotypes between Stage I and Stages II-IV, and Stages I + II and Stages III + IV. It is further shown in Table 5 that the presence of invasion in more than half of the myometrium, pelvic, and para-aortic lymph node metastases were not related to the GST genotypes.

Discussion

Genetic variation in susceptibility to chemical carcinogens among individuals is one of the main contributing factors leading to cancer development among human beings. Genetic variations of genes which produce enzymes that play a role in intracellular metabolism such as GST have shown to lead susceptibility in the development of various cancers [6-8]. The mu and theta classes of GST isoenzymes (GSTM1 and GSTT1, respectively) have a common and broad range of substrate specificities, and they detoxify the reactive metabolites of benzo-a-pyrene and other polycyclic aromatic hydrocarbons [32]. Expression of GST pi enzyme was found to correlate with the histological grade and chemoresistance of endometrial carcinoma [33]. The null genotype developing from homozygote deletion of GSTM1 or GSTT1 genes was frequently observed in lung, colorectal, and bladder cancers [20, 21, 34-37]. GSTP1 polymorphism was found in association with colorectal [38] and breast cancer [39].

In this population-based case-control study, the authors investigated the role of the GSTM1, GSTT1, and GSTP1 gene polymorphisms in endometrial cancer patients of Caucasian origin. When compared with other studies, this current study performed in a Caucasian population showed similar genotype and allelic frequency distribution with the previous studied populations [19, 40]. The presence of the GSTM1 and GSTT1 null genotype did not influence the risk of developing endometrial carcinoma in this study population. Also, the authors showed that GSTP1 genotype frequencies did not differ significantly between patients with endometrial cancer and the control group. In addition, the presence of GSTP1 G allele did not influence the risk of developing endometrial cancer in patients with endometrial cancer when compared to the control group. Nevertheless, when the authors considered that the etiology of most commonly occurring cancers could not be simply explained by allelic variability at a single locus, the significance of attributed risk could be reached when interactions of polymorphisms of aforementioned genes with each other and the other identified etiological factors were considered. From this point of view, the authors calculated combined risk of GSTM1 and GSTP1 genotypes with GSTT1 genotypes between patient and control groups. However, the authors could not demonstrate any specific genotype combination that increased the risk of endometrial carcinoma development. Additionally, environmental and genetic factors including the presence of DM, HT, smoking, and history of endometriosis did not have an impact on endometrial cancer development in this study population.

The authors did not find any association between GSTM1, GSTT1, and GSTP1 polymorphisms and histological subtypes of the endometrial carcinoma. There was also no association between polymorphisms of all three GST genes and histological grade of the endometrial cancer. Frequencies of GSTM1 and GSTT1 genotypes were not statistically different between patients with Stage I+II and Stage III+IV, early stage (Stage I), and advanced stage (Stage II-IV) endometrial cancer, whereas the frequencies of GSTP1 AA genotype and GSTP1 GG genotype were statistically significant between patients with early and advanced stage disease. There were no other statistical differences in genotype frequencies concerning deep myometrial invasion, pelvic, and para-aortic lymph node metastasis between cases and the controls.

Previous studies concerning the role of GST genes in endometrial cancer have yielded mixed results. There have been conflicting studies regarding the role of GSTM1, GSTT1, and GSTP1 polymorphisms in endometrial carcinoma. Ueda *et al*. found that GSTT1 null genotype was dominant in endometrial carcinoma cells and also the frequency of GSTM1 null genotype was not associated with increased risk for endometrial carcinoma development [27]. The study by Chan *et al*., reported a strong association between endometrial adenocarcinoma and GSTP1 AG polymorphism in a Chinese cohort [40]. However, interestingly they found no statistical difference in frequency of GSTP1 GG genotype. Therefore, the latter result was contradictory to the expected outcome, as in this hypothesis much more abolished enzyme production should entail unconjugated substrates in target tissue which is thought to increase endometrial cancer development risk. In another study by Chan *et al*., post-transcriptional modification of GSTP1 gene in endometrial carcinoma patients was investigated [41]. In that study, they found statistically significant correlations between reduced GSTP1 mRNA and enzyme expression and hypermethylation of the GSTP1 gene in endometrial carcinoma. The researchers concluded that GSTP1 gene expression was altered at the transcriptional level. When the present findings are interpreted and compared with those results, it is also possible that altered expression of GSTP1 enzymes may be a result from factors other than polymorphisms of the GSTP1 gene.

Conclusion

The authors did not find any association between GSTM1, GSTT1, and GSTP1 polymorphisms and histological subtype, histological grade, lymph node status and clinical stage of endometrial carcinoma. Furthermore, there is no evidence supporting the clinical importance of GSTM1, GSTT1, and GSTP1 polymorphisms and increased risk of endometrial carcinoma development in Caucasian population.

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Depth of glandular crypts and its involvement in squamous intraepithelial cervical neoplasia submitted to large loop excision of transformation zone (LLETZ)

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Summary

Background: The authors aimed to confirm the depth of six mm in order to achieve an optimal eradication of the lesion. *Materials and Methods*: This is a retrospective observational study of 94 cervical surgical pieces from women aged 17 to 22 years with a cyto-colpo-histopathological diagnosis of high-grade squamous cervical intraepithelial neoplasia (CIN II and/or CIN III) submitted to large loop excision of transformation zone (LLETZ). The glandular crypts and margins, both exposed or not to CIN, were assessed. The compromise and the maximum depth of the glandular crypts were noticed. *Results:* After LLETZ, 23 (24.47%) cases presented a neoplasic impairment of endocervical margin and ten (10.64%) of the ectocervical margin. The largest noticed crypt measured 4.500 mm and the shortest 0.100 mm, with an average of 2.148 mm. *Conclusions:* Squamous CIN more frequently show the exposure of surgical margins to LLETZ. The deeper location of glandular crypts in the cases studied was 4.500 mm, while the largest neoplastic extension was 3.000 mm.The therapeutic method depends on this knowledge.

Key words: Glandular crypt; Cervical intraepithelial neoplasia; LLETZ; Cervix.

Introduction

The cervix is lined by two different epithelia: squamous and columnar: the squamous epithelium is pluristratified and nonkeratinized and the monostratified columnar epithelium comprises of a single layer of muco-secreting cylindrical cells, and runs a sinuous trajectory, delimitating structures named glandular crypts. The transformation zone is a squamous epithelium and comprises of the area between the original squamous epithelium and the glandular one as a consequence of a metaplasic process

Cervical epithelia, more specifically its transformation zone, might shelter cervical intraepithelial neoplasias (CIN), also known as low- mid- and high-grade lesions, at any portion of its extension. The knowledge of the cervical lining structure leads to the compliance with rigid precepts in choosing the destructive treatment for low-grade intraepithelial lesions. Since high-grade intraepithelial lesions, have a higher potential of evolution to invasive lesions, due to the larger action on glandular crypts in its depth, and an option is made for the surgical excision of loops with large loop excision of transformation zone (LLETZ) [1-3].

In a histopathological analysis conducted by Anderson and Hartley, the depth of glandular crypts, both free or presenting neoplasia, was measured. Eighty-five percent of the pieces presented involvement of crypt by neoplasia, and their depth ranged from 1.24 to 5.22 mm. For this reason, authors should consider a depth of six mm in order to achieve an optimal and safe eradication of the lesion [4].

The authors aimed in assessing the exposure of crypts by CIN, in order to confirm or not these findings.

Materials and Methods

This is a retrospective observational study performed at the Gynecologic Diseases Prevention Center, Gynecology Department of Escola Paulista de Medicina, Federal University of São Paulo - EPM-UNIFESP, from August 2019 to June 2011.

Many surgical cervical pieces originated from women with a cyto-colpo-histopathological diagnosis of high-grade squamous intraepithelial neoplasia (HSIL) (CIN II and/or III). The clinical findings were obtained from an analysis of clinical records and these women were submitted to large loop exeresis of transformation zone or conization as a complementary diagnostic conduct, after a colposcopic analysis and conducted biopsy. The entire project was presented to the Research Ethics Committee of Hospital São Paulo - UNIFESP, and was approved under the number 0178/09.

Upon study of histopathological views, stained through the Hematoxilin and Eosin (H&E) method, [5] the glandular crypts and margins, both exposed or not to high-grade squamous intraepithelial neoplasia, were assessed and were divided into two groups of analysis. Through the histopathological measuring technique with a Breslow microscopic rule, the glandular crypt depth in each group was measured. The exposure of surgical margins was also noticed in each group and was related to the histopathological diagnosis and to the maximum depth of glandular crypt.

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Results

Only 94 pieces out of the initially-selected 104 products of LLETZ, could be measured. The other pieces presented tangential sections, which rendered it impossible to take precise measurements. The patients age ranged from 17 to 72 years, with an average of 33.7 years The ethnic groups were as follows: 38 (40.4%) women were white, 54 (57.4%), afro-descendents, and the remaining two were Indians. The initial cytopathological results were the following: 55 (58.51%) of cases were compatible with high-grade lesion/invasive spinocellular carcinoma (ISC); low-grade lesions occurred in 13 (13.82%) cases, and ISC were seen in three (3.19%) cases.

In 56 (59.57%) patients, the abnormal colposcopic aspect noticed was unique, with the associated aspects noticed in other 36 (38.30%) women. After LLETZ, the definite histopathological diagnosis was of grade III intraepithelial neoplasia (CIN III) in 42 (44.68%) cases; 30 (31.92%) cases, the result was grade-II intraepithelial neoplasia (CIN II); 22 cases comprised of six (6.38%) cases with spinocellular carcinoma, and 16 (17.02%) cases with grade-I (CIN I) intraepithelial neoplasia.

In the 48 cases with initial diagnosis of CIN II, an agreement was noticed with the LLETZ diagnosis in 23 (47.92%) cases and a final diagnosis of CIN III in 18 (37.50%) cases. In 21 (67.74%) biopsy cases of CIN III, histopathological agreement in the surgical piece was achieved.

In the 94 operated cases, 23 (24.47%) presented a neoplasic impairment of endocervical margin, and ten (10.64%) cases of the ectocervical margin. Both surgical margins were exposed in eight (8.51%) cases, and the assessment was not possible in one CIN III case. The higher exposure index of surgical margins was noticed in CIN III cases (20 out of 40 cases), as seen in Table 1. This Table allows an assessment of the higher impairment of margins in CIN II, CIN III, and ISC cases ($p = 0.007$).

Ninety-four cases were submitted for glandular crypt measurement. The largest noticeable crypt measured 4.500 mm, and the shortest 0.100 mm, with an average of 2.148 mm (Table 2).

No significant statistics were found among the crypt extension, neoplasic extension, and histopathological diagnoses in LLETZ. In 53 (56.38%) cases, the neoplasic extension into the glandular crypts was noticed. In Table 3, the histological types (CIN II, III, and ISC) more often presented the neoplasic extension for crypts ($p = 0.001$).

No significant statistics were seen in comparing the impairment of surgical margins with the neoplasic extension for crypts (Table 4).

Table 1. — *Relationship between histopathological diagnosis (LLETZ) and neoplastic exposure of surgical margins in 94 patients bearing intraepithelial neoplasia, submitted to LLETZ.*

Cytopathological diagnosis	Impairment of surgical margin											
	Endocervical		Ecsocervical		Both		Not involved		No assessment		Total	
	N	%	N	%	N	%	N	%	N	%	N	%
CIN I	1	6.25	0	0.00	1	6.25	14	87.50	0	0.00	16	100.00
CIN II	9	30.00	4	13.33	2	6.66	15	50.00	0	0.00	30	100.00
CIN III	11	26.19	6	14.28	3	7.14	21	50.00	1	2.38	42	100.00
ISC	2	33.33	0	0.00	1	16.66	3	50.00	0	0.00	6	100.00
Total	23	24.47	10	10.64	7	7.45	53	56.38	1	1.06	94	100.00

CIN I: intraepithelial neoplasia grade I; CIN II: intraepithelial neoplasia grade 2; CIN III: intraepithelial neoplasia grade 3; ISC: invasive spinocellular carcinoma. Chi-square test (Pearson) $\chi^2 = 7.339$ $p = 0.007$. N = cases number.

Table 2. — *Assessment of crypts depth in 92 cases and 60 cases of neoplastic extension into crypts, in 94 patients bearing intraepithelial neoplasia, submitted to LLETZ.*

Depth (mm)	Glandular crypts	
	Neoplastic extension (60 cases)	Maximum depth (94 cases)
Larger	3.000	4.500
Smaller	0.100	0.700
Medium	0.950	2.200
Average	1.075	2.148
Standard deviation	0.703	0.845

Table 3. — *Relationship between histopathological diagnosis (LLETZ) and exposure of crypts in 94 patients bearing intraepithelial neoplasia, submitted to LLETZ.*

Cytopathological Diagnosis	Neoplastic extension into crypt					
	Present		Absent		Total	
	N	%	N	%	N	%
CIN I	2	12.50	14	87.50	16	100.00
CIN II	18	60.00	12	40.00	30	100.00
CIN III	28	66.66	14	33.33	42	100.00
ISC	5	83.33	1	16.66	6	100.00
Total	53	56.38	41	43.62	94	100.00

CIN I: intraepithelial neoplasia grade I; CIN II: intraepithelial neoplasia grade 2; CIN III: intraepithelial neoplasia grade 3; ISC: invasive spinocellular carcinoma. Chi-square test (Pearson) $\chi^2 = 11.509$ $p = 0.001$.

Table 4. — *Relationship between neoplastic extension into glandular crypts and exposure of surgical margin in 93 cases (one of them not assessed, in view of an improper piece).*

Exposure of Crypts	Exposure of surgical margin								Total	
	Endocervical		Ectocervical		Both		Free			
	N	%	N	%	N	%	N	%	N	%
Present	14	26.41	7	13.21	4	7.55	28	52.83	53	100.00
Absent	9	22.50	3	7.50	3	7.50	25	62.50	40	100.00
Total	34	25.73	10	10.75	7	7.53	53	56.00	93	100.00

Chi-square test (Pearson) $\chi^2 = 0.870$ $p = 0.351$.

Discussion

In 94 patients with CIN submitted to LLETZ, a prevalence of high-grade squamous lesions was noticed in women living their fourth decade of life. These findings are similar to those referred to by other authors [1, 2]. With regards to colposcopy, most of the cases presented a unique and abnormal aspect.

Sixteen out of 94 cases presented negative cytology, but the conducted biopsies revealed CIN II involved in nine cases and CIN III in five cases. The histopathological results of biopsies agreed with those from a cytopathological exam compatible with HSIL.

The LLETZ conduct had grounds on the diagnostic agreement of HSIL, the cytohistopathological disagree-

ment, and/or the persistence of CIN I. Similarly to literature, a diagnostic agreement among the products of biopsies and LLETZ was reached, mainly in CIN II/III case [1]. The CIN III cases prevailed among those allowing the identification of exposed surgical margins. These facts suggest that CIN III lesions have larger dimensions and are frequently more involved in the endocervical channel. The measurement of columnar epithelium crypts were smaller compared to those referred by Anderson and Hartley [4]. The agreement of neoplasic extension to glandular crypts, the exposure of surgical margins, and the higher severity of lesion became evident.

Conclusion

In view of their features of dimension and location, CINs more frequently show exposure of surgical margins to LLETZ. Likewise, they extend more often into the glandular crypts. The deeper location of glandular crypts in the cases studied was 4.500 mm, while the largest neoplastic extension was 3.000 mm. The choice of the therapeutic method adopted depends on this knowledge.

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Atypical endometrial lesions: hysteroscopic resection as an alternative to hysterectomy

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Summary

Background: Endometrial hyperplasia is a precursor to endometrial carcinoma: the risk of progression to invasive endometrial cancer is increased in postmenopausal women and much more in cases of atypical endometrial hyperplasia (25%-30%). In addition, in 12.7% to 42.6% of cases according to various studies, endometrial cancer coexists in patients with diagnosis of atypical endometrial hyperplasia. The aim of this study was to evaluate the correlation between radical hysteroscopic resection of atypical endometrial lesions and the histopathological examination of the uterus. *Materials and Methods:* The authors collected 25 patients referring to the Department of Woman and Child Health, in the University of Padua (Italy) from January 2008 to June 2012, undergoing hysteroscopic resection for atypical polyps and focal atypical endometrial hyperplasia, and following hysterectomy within 30 days. Average age, menopausal status, hormone replacement therapy, body mass index (BMI), presence of hypertension and diabetes, and taking tamoxifen were reported. *Results:* After hysteroscopic resection in all patients atypical polyps and focal endometrial hyperplasia were confirmed. The hystopathologic evaluation of the uterus reported: in only two (8%) cases, the persistence of atypical endometrial lesion, whereas in 23 (92%) cases the endometrial tissue was negative for atypia or malignancy. *Conclusions*: Radical endometrial resection by hysteroscopy may serve as an alternative to hysterectomy in selected patients with atypical focal endometrial lesions, not only in fertile women, but also in patients who refuse hysterectomy or present high anesthesiologic and surgical risks, regardless of the risk of recurrence, and with the necessity of undergoing hysteroscopic close follow-up.

Key words: Resectoscope; Atypical endometrial hyperplasia; Atypical endometrial polyps; Conservative management.

Introduction

Endometrial hyperplasia represents a pre-malignant lesion of the endometrium which has a greater risk of progression into an invasive endometrial cancer in postmenopausal women who may often present atypical lesions [1]. Atypical hyperplasia shows greater short-term risks of progression into endometrial cancer within four years [2] in approximately 25%-30% of cases, whereas simple or complex hyperplasia without atypia is likely to progress in < 5%, within ten years [3, 4]. Without persistent estrogen stimulation, endometrial hyperplasia without atypia can, in many cases, revert spontaneously to a normal endometrium. In addition, according to literature, 12.7% to 42.6% of the cases with endometrial cancer also present atypical endometrial hyperplasia lesions [5]. Endometrial hyperplasia or polyps do not always cause symptoms, although abnormal uterine bleeding is common [6]. More than 90% of endometrial malignancies occur in women over 50 years who suffer from abnormal uterine bleeding. An early diagnosis is therefore highly recommended [7]. Curettage of the uterine cavity was considered for years the gold standard for abnormal uterine bleeding, although most focal lesions in the uterine cavity remained undetected (58% polyps, 50% hyperplasia, 60% atypical hyperplasia, and 11% cancers) [8], yielding false negative rates of 3% to 7% [9]. The risk that an atypical endometrial lesion may transform itself into an endometrial carcinoma involved performing a hysterectomy, even in young women. Currently, hysteroscopic resection has become the novel route for removing any intrauterine lesion, thus not only benign pathologies, but also minimal malignant lesions regardless of the risk. The aim of this study was to evaluate the hysteroscopic resection as an alternative to hysterectomy to completely remove an atypical endometrial focal lesion and compare it to the histopathology of the uterus.

Materials and Methods

In the period from January 2008 to June 2012, out of 2,900 women referred to Hysteroscopic Unit of the Department of Health of Woman and Child of the University of Padua (Italy), for abnormal uterine bleeding and endometrial thickening in postmenopause, the data of 25 (0.8%) patients with a hystopathological diagnosis of atypical polyp or focal atypical endometrial hyperplasia were collected.

An office hysteroscopy with endometrial biopsy was performed, without anesthesia and/or analgesia, in the early days after the menstrual cycle, according to the method previously described in other papers by the same authors [10-12].

Twenty-five patients with atypical endometrial lesions underwent operative hysteroscopy as an inpatient day surgery under general anesthesia using a nine-mm resectoscope with a 12° forward-oblique lens, with a monopolar 90° loop, and glycine as distension medium, with the aim of entirely removing the endometrial lesions. In premenopausal women the procedure was performed during the proliferative phase of the menstrual cycle. The same patients were then submitted to total laparoscopic hysterectomy [13] within 30 days.

Specimens removed by hysteroscopic resection (endometrial polyps and focal endometrial areas) and by laparoscopic surgery (uterus), were sent to the Institute of Pathological

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Anatomy of the University of Padua for histopathological examination.

Patient age, body mass index (BMI), menopausal status, hormone replacement, tamoxifen therapy, hypertension, and diabetes were recorded. Patients exceeding a year since the last menstrual period and age above 40 years were defined as postmenopausal.

Results

Twenty-five patients were included in the study. Mean age: was 60.8 years (range 39 – 74), and the demographic characteristic are described in Table 1.

Only seven (28%) women had hormone replacement therapy with average of 13.5 years (range 1-25) and three (12%) patients with previous breast cancer had tamoxifen (20 mg/day) for five years. No patient had a family history of bowel, breast, and endometrial cancer.

The indications for office hysteroscopy were: abnormal uterine bleeding in 12 (48%) patients and postmenopausal endometrial thickening in 13 (52%) patients (Table 2).

The histopathological diagnosis of atypical polyp was confirmed in 16 (64%) women, and in nine (36%) women, atypical focal endometrial hyperplasia after resectoscopic surgery was found. Moreover, in case of polyps, an endometrial biopsy at the basal area of the polyp was added and only in three (12%) cases was an additional transcervical resection of the endometrium performed.

No complications such as uterine perforation, excessive absorption of distension medium, endometritis or failed procedure occurred.

After laparoscopic hysterectomy, histopathological examination of the uterus did not show the persistence of endometrial atypia in 23 (92%) cases, whereas in two (8%) cases endometrial atypical tissue was still present. No case of endometrial carcinoma was reported. There were no intra- or post-operative complications after laparoscopic surgery.

Table 1. — *Demographic data of 25 patients*.

	Number	Percentage
Premenopausal	7	28%
Postmenopausal	18	72%
Hormone replacement therapy	7	28%
Nulliparous	5	20%
Multiparous	20	80%
Overweight (BMI ≥ 25 - < 30 Kg/m^2)	10	40%
Obesity (BMI ≥ 30 Kg/m^2)	8	32%
Hypertension	10	40%
Diabetes mellitus	2	8%
Family history of bowel/breast/ endometrial cancer	0	0%
Tamoxifen (20 mg/day)	3	12%

Table 2. — *Indications to hysteroscopy*.

	Abnormal uterine bleeding	Endometrial thickening (≤ 4 mm)	Total
Premenopause	7 (28%)	–	7 (28%)
Menopause	5 (20%)	13 (52%)	18 (72%)

Discussion

The most important risk factor of progression of endometrial hyperplasia into endometrial cancer is more closely related to the presence of cytological atypia. There are still debates regarding the possibility of conservative management in these patients. The risk of progression to endometrial adenocarcinoma for atypical hyperplasia occurs in 25%-30%, with a time span of four years [2, 14]. Previous studies reported that, in cases of untreated atypical hyperplasia, the regression, persistence, and progression to endometrial carcinoma over a mean period of 13.4 years was 60%, 17%, and 23%, respectively [5].

In women who have completed their families or who are postmenopausal, the therapy of choice for atypical endometrial hyperplasia is hysterectomy, whereas in younger patients conservative medical or minimally invasive surgical treatments could be offered.

Currently there are no standardized treatment protocols, but the choice depends on the severity of the lesion, the patient's age, medical history, and the preferences of the patient. Medical treatment in young women with diffuse atypical endometrial hyperplasia could lead to regression of the lesion; instead surgical treatment, such as hysteroscopic resection, can be suggested in cases of atypical lesions as polyp or focal endometrial hyperplasia, even if hysteroscopic surveillance is mandatory.

Some authors have conducted studies with experimental protocols with hysteroscopic resection as conservative treatment for atypical polyps, with benign polyp base, and surrounding endometrium, both in young patients who wish to preserve their fertility, and in postmenopausal patients not undergoing hysterectomy under general anesthesia because of high anesthesiologic risk for important co-morbidity. In all patients, the follow-up period was five years, with an outpatient hysteroscopic assessment with endometrial sampling every six months during the first two years and subsequently every year thereafter [15]. In the fertile group, the patients were divided in two subgroups: the first in which the patients were subjected to insertion of levonorgestrel intrauterine device (LNG-IUD) and the second as control group; after five-years follow-up, there was no significant difference between women in the two subgroups. At the end of the trial, there were no recurrences of atypical polyps in both subgroups [16].

The results of the present retrospective study showed that in women treated with resectoscopic excision of atypical endometrial lesions, the therapeutic efficacy occurred in 92% of the patients and only in 8% of patients there was persistent atypical lesions. The authors believe that the limit of this procedure occurs when the lesion is close to the antrum of the uterine tube with a thickness < one cm because, beyond the excision of the lesion, there is a high risk of uterine perforation while performing a large biopsy of the surrounding tissue.

In a recent study [17], six young women with focal endometrial adenocarcinoma (grade G1) were conservatively treated with hysteroscopic resection followed by hormone therapy with megestrol acetate (160 mg/day) for six months. The radical excision of the lesion was confirmed by the absence of atypia in the surrounding tissue, while medical therapy had only the aim of consolidation.

The conservative management of Stage I endometrial carcinoma in young women is accepted as a reasonable short-term alternative to definitive surgical treatment. The risk of disease progression during conservative management is 5%-6% [18], while in cases of persistent disease, total hysterectomy is mandatory.

Considering the findings from this retrospective study, which are in accordance with others present in the literature regarding hysteroscopic resection for atypical endometrial hyperplasia, the authors can state that this procedure is safe, and a skillful surgeon is able to completely remove the intracavitary lesions. Furthermore this procedure could be suggested as a treatment alternative to hysterectomy in fertile women who desire to become pregnant or when hysterectomy is refused, or with high anesthesiologic and surgical risks. In these selected patients, it is necessary to perform a strict follow-up through an office hysteroscopy with endometrial biopsy.

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HLA DRB allele polymorphisms and risk of cervical cancer associated with human papillomavirus infection: a population study in China

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Summary

Objective: Persistent infection with high-risk human papillomavirus (HPV) is the main cause of cervical cancer. Environmental, behavioral, and ill-defined genetic factors have also been implicated in the pathogenesis of this disease. To determine whether human leukocyte antigen (HLA) DRB alleles are associated with cervical cancer and HPV infections in the Chinese population, HLA genotypes were examined in 69 cervical cancer patients and 201 controls. *Materials and Methods:* Polymorphisms in HLA-DRB genes were genotyped using oligoneucleotide arrays, and the magnitude of associations was determined by logistic regression analysis. *Results:* HLA-DRB1*13 (OR = 4.01 95% CI, 1.703 - 9.442) and HLA-DRB1*3(17) (OR = 2.661 95% CI, 1.267 - 5.558) were associated with an increased risk of cervical cancer, and DRB1*09012 (OR = 0.182, 95% CI, 0.079 - 0.418 and DRB1*1201 (OR = 0.35 95% CI, 0.142 - 0.863 were associated with a decreased risk. The risk associations of HPV infection were increased in women carrying the HLA-DRB1*09012 (OR = 1.924; 95% CI, 1.08 - 3.427) and DRB3(52)*0101 (OR = 7.527 95% CI, 0.909 - 62.347) alleles. Among cervical cancer patients, the risk associations differed between HPV positive and negative cases for several alleles; increased risk of cervical cancer was associated with DRB3 (52)*02/03 (OR, 12.794; 95% CI, 5.007 - 32.691) and DRB1*3(17) (OR = 3.48; 95% CI, 1.261 - 9.604), and decreased risk was associated with DRB1*09012 and DRB5(51)*01/02. Furthermore, HPV16-containing cervical cancer cases differed from non-HPV16 subjects in their positive association with DRB1*1501 (OR = 4.173; 95% CI, 1.065 - 16.356) and DRB5(51)*0101/0201, and their negative association with DRB4(53)*0101 (OR = 0.329; 95% CI,0.122 - 0.888). *Conclusions:* The present results provide further evidence that certain HLA class II allele polymorphisms are involved in the genetic susceptibility to cervical cancer and HPV infection in the Chinese population from an area with a high incidence of this neoplasia.

Key words: Cervical cancer; Human leukocyte antigens; Human papillomavirus.

Introduction

Cervical cancer (CC) is the second most common cancer among women worldwide. Although population wide screening in most countries has led to a remarkable reduction in the incidence and mortality of CC, it remains a major global health burden with approximately 500,000 new cases and 250,000 cancer-related deaths each year. Human papillomaviruses (HPV) have been identified as the major etiological factor in cervical carcinogenesis [1]. However, most patients with HPV-associated lesions such as cervical intraepithelial neoplasm (CIN) will remain stable or spontaneously regress over time. Only a small proportion of women with persistent oncogenic HPV infection develop malignant cervical lesions. Although oncogenic HPV genes are capable of immortalization and can contribute to the process of transformation, not all non-invasive lesions progress to the full malignant phenotype [2]. Therefore, other genetic or epigenetic events or individual immune responses to HPV infection are likely to be involved in cervical carcinogenesis. The combination of such factors may lead to a series of molecular events that result in the evolution of intraepithelial and invasive disease.

Experimental and clinical evidence demonstrate that the immunological and genetic background of the host plays an important role in the outcome of HPV associated diseases. The human leukocyte antigen (HLA) class I and II molecules play a critical role in the process by which HPV peptides are presented to T-cells. High-affinity engagement of a T-cell receptor with a HPV peptide-HLA complex and a co-stimulatory signal is necessary to activate a T-cell response. HLA Class I molecules (HLA-A,-B,-C) are found in most nucleated cells and present peptides derived from the cytosol to cytotoxic T-cells. HLA class II molecules (HLA-DR,-DQ,-DP) are found in antigen-presenting cells (e.g., dendritic cells and macrophages) and present peptides degraded in intracellular vesicles to helper T-cells [3]. An effective immune response may require optimal peptide presentation by both class I and II molecules to activate efficient helper and effector T-cell responses to HPV. Subtle changes or impairment in T-cell responses may allow escape from immune surveillance, induction of immune allergy, or tolerance to HPV peptides [4].

Although associations between specific HLA alleles and cervical neoplasia have been reported in numerous studies, the alleles and haplotypes associated with disease

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Table 1. — *Relative risk of cervical cancer associated with HLA class II DRB alleles.*

Gene type	Controls (n = 201)	%	Cases (n = 69)	%	OR-adjusted*	95% CI	*p* value
DRB1*0101	11	5.47	5	7.25	1.393	0.466-4.165	0.552
DRB1*04	41	20.4	22	31.88	1.827	0.991-3.367	0.052
DRB1*0701	15	7.46	3	4.35	0.626	0.174-2.249	0.575
DRB1*08	44	21.89	20	28.99	1.518	0.816-2.825	0.186
DRB1*09012	77	38.31	7	10.14	0.182a	0.079-0.418	0
DRB1*1001	9	4.48	0	0	1.047	1.016-1.027	0.118
DRB1*11	25	12.43	8	11.59	1.041	0.446-2.431	0.925
DRB1*1201	43	21.39	6	8.69	0.35a	0.142-0.863	0.018
DRB1*13	18	8.96	14	20.29	2.588	1.209-5.537	0.012
DRB1*13-1	3	1.49	1	1.45	0.007	0.112-10.771	0.935
DRB1*13-2	11	5.47	13	18.84	4.01b	1.703-9.442	0.001
DRB1*1301	4	1.99	0	0	1.02	1-1.041	0.575
DRB1*1405	11	5.47	0	0	1.058	1.023-1.094	0.071
DRB1*1406	18	8.96	5	7.25	0.82	0.292-2.301	0.706
DRB1*15	38	18.91	16	23.19	1.295	0.668-2.509	0.443
DRB1*1601	5	2.49	0	0	1.026	1.003-1.048	0.333
DRB1*3(17)	19	9.45	15	21.74	2.661b	1.267-5.558	0.008
DRB3(52)*0101	8	3.98	3	4.35	1.097	0.283-4.256	1
DRB3(52)*02 /03	107	53.23	31	44.93	0.717	0.414-1.241	0.234
DRB4(53)*0101	120	59.7	32	46.38	0.584	0.337-1.013	0.054
DRB5(51)*0101/0201	43	21.39	16	23.19	1.109	0.577-2.131	0.756

* adjusted for age; a: $p < 0.05$; b: $p < 0.01$.

Table 2. — *Relative risk of HPV infection associated with HLA class II DRB alleles in control subjects.*

HLA-DRB	HPV positive subjects (n = 100)	HPV negative subjects (n = 101)	OR-adjusted*	95% CI	*p* value
DRB1*0101	4	7	0.56	0.159-1.974	0.361
DRB1*0401	20	21	0.952	0.479-1.892	0.889
DRB1*0701	6	9	0.652	0.223-1.906	0.432
DRB1*0801	21	23	0.901	0.462-1.76	0.761
DRB1*09012	46	31	**1.924**	1.08-3.427	0.026
DRB1*1001	4	5	0.8	0.208-3.07	0.745
DRB1*1101	15	10	1.606	0.684-3.769	0.273
DRB1*1201	18	25	0.667	0.338-1.319	0.234
DRB1*13-1	1	2	0.5	0.045-5.604	0.567
DRB1*13-2	8	3	2.841	0.731-11.035	0.117
DRB1*1301	1	3	3.031	0.31-29.639	0.317
DRB1*1405	4	7	0.56	0.159-1.974	0.361
DRB1*1406	9	9	1.011	0.384-2.662	0.982
DRB1*15	21	17	0.761	0.375-1.548	0.45
DRB1*1601	3	2	1.531	0.25-9.364	0.683
DRB1*3(17)	7	12	0.558	0.21-1.482	0.237
DRB3(52)*0101	7	1	7.527	0.909-62.347	0.035
DRB3(52)*02/03	49	58	0.712	0.408-1.242	0.231
DRB4(53)*0101	64	56	1.429	0.811-2.517	0.216
DRB5(51)*0101/0201	24	19	1.429	0.811-2.517	0.37

* adjusted for age.

Table 3. — *Relative risk of HPV-containing CC and control group associated with HLA II DRB alleles.*

Alleles	HPV positive subjects Cases (n = 53)	Control (n = 100)	OR-adjusted*	95% CI	*p* value
DDRB1*0101	5	4	2.5	0.642-9.737	0.277
DRB1*04	13	20	1.3	0.587-2.878	0.517
DRB1*0701	3	6	0.94	0.225-3.919	1
DRB1*08	18	21	1.935	0.919-4.074	0.08
DRB1*09012	5	46	0.122	0.045-0.333	0
DRB1*1001	0	4	1.024	1.001-1.084	0.299
DRB1*11	7	15	0.862	0.328-2.266	0.764
DRB1*1201	6	18	0.582	0.216-1.567	0.28
DRB1*13-1	1	1	1.904	0.117-31.06	0.574
DRB1*13-2	9	8	2.352	0.85-6.509	0.093
DRB1*1301	0	1	1.01	0.99-1.03	0.654
DRRB1*1405	0	4	1.042	1.001-1.084	0.299
DRRB1*1406	5	9	1.503	0.334-3.319	0.572
DRB1*15	15	21	1.485	0.689-3.199	0.311
DRB1*1601	0	3	1.031	0.996-1.067	0.552
DRB1*3(17)	11	7	3.48	1.261-9.604	0.012
DRB3(52)*0101	3	7	0.797	0.197-3.218	0.75
DRB3(52)*02 /03	26	7	12.794	5.007-32.691	0
DRB4(53)*0101	20	49	0.631	0.32-1.245	0.183
DRB5(51)*0101/0201	15	64	0.222	0.108-0.458	0

* adjusted for age.

have varied from study to study in different populations. Some studies have suggested a negative association between HLA class II DRB1*13 alleles and CC [5]. Other studies have failed to confirm this association, but have suggested a positive association between HLA class II DRB1*1501, DQB1*0602 and disease risk [6]. Interestingly, although reports have often been inconsistent with respect to the specific HLA alleles positively associated with cervical disease, many of these studies consistently reported a reduction in risk of disease associated with HLA class II DRB1 alleles.

In the present study, the authors conducted gene chip HPV typing to assess the risk of squamous cell cervical cancer associated with class II HLA-DRB loci in China. Polymorphisms of HLA-DRB genes were analyzed including DRB1-1 DRB1-3 DRB1-4 DRB1-7 DRB1-8 DRB1-9 DRB1-10 DRB1-11 DRB1-12 DRB1- DRB1-14 DRB1-15, DRB1-16, DRB3, DRB4, and DRB5 in a case-control study of women from the county of WuFeng in the Hubei province. This region has one of the highest incidence rates of CC in China.

Table 4. — *Relative risk of cervical cancer and HPV16 associated with HLA class II DRB alleles.*

Gene type	HPV 16 positive cases (n = 40)	HPV 16 negative cases (n = 29)	OR-adjusted*	95% CI	*p* value
DRB1*0101	4	1	3.111	0.329-29.407	0.389
DRB1*04	10	12	0.472	0.169-1.321	0.15
DRB1*0701	2	1	1.474	0.127-17.071	1
DRB1*08	14	6	2.064	0.681-6.256	0.196
DRB1*09012	2	5	0.253	0.045-1.407	0.122
DRB1*1001	0	0	0	0	0
DRB1*11	4	4	0.694	0.159-3.041	0.712
DRB1*1201	4	2	1.5	0.256-8.794	1
DRB1*13	7	7	0.667	0.205-2.166	0.499
DRB1*13-1	1	0	0.975	0.928-1.025	1
DRB1*13-2	6	7	0.555	0.165-1.87	0.338
DRB1*1301	0	0	0	0	0
DRB1*1405	0	0	0	0	0
DRB1*1406	3	2	1.095	0.171-7.008	1
DRB1*15	13	3	**4.173**	1.065-16.356	0.031
DRB1*1601X	0	0	0	0	0
DRB1*3(17)	9	6	1.113	0.347-3.569	0.857
DRB3(52)*0101	0	3	1.115	0.986-1.262	0.07
DRB3(52)*02/03	19	12	1.282	0.488-3.364	0.614
DRB4(53)*0101	14	18	**0.329**	0.122-0.888	0.026
DRB5(51)*0101/0201	13	3	**4.173**	1.065-16.356	0.031

* adjusted for age.

Materials and Methods

Samples

A total of 69 CC cases and 201 out of 1,023 controls were randomly selected from an epidemiological study of CC and HPV infection conducted in mid-western China. The controls were women with normal or non-dysplastic Pap smears. Epidemiological data from all subjects were obtained during a standardized interview carried out by a trained nurse using a structured questionnaire. Information regarding socio-demographic variables, education, sexual behavior, diet, personal hygiene habits, and reproductive history was obtained. The study was approved by the local ethics committee.

After the interview, all subjects were asked to provide a sample of peripheral blood, and DNA was extracted and stored at -80 °C. Aliquots of the purified DNA were used for HLA class II allele typing and HPV detection. A cervical cellular specimen was collected from each control using a cytobrush, and tumor biopsies were obtained from all cancer patients. All specimens were prepared and submitted for cytological or histological examination.

HLA typing

All specimens from CC cases and controls were typed for HLA-DRB1 DRB3 DRB4 DRB5 DRB6 DRB7 and DRB9 genes using a HLA -DRB gene typing chip (UnitedGene, Shanghai, China). The identification and naming of HLA-DRB genes used in this study followed the 12th International Histocompatibility Workshop and Conference recommendations.

HPV typing

HPV detection and typing were performed by polymerase chain reaction (PCR) based amplification of a 450 bp segment in the L1 viral gene with MY09 and MY11 primers. PCR products were dot-blotted onto a nylon membrane and hybridized with individual 32P-labeled oligonucleotide probes specific for HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-59, 66, and 68. A 268 bp fragment of the b-globin gene was amplified to ensure DNA quality. A negative control tube containing all PCR reagents except template DNA was included in all PCR reactions, and DNA from HeLa cells that harbor HPV18 integrated into the host genome was used as a positive control.

Statistical analysis

The distribution of specific HLA genotypes in CC cases and controls was compared using the 2 test, and the HLA allele frequencies for each group were ascertained. Comparisons of exposure profiles between cases and controls were performed by 2 tests for independent samples. The magnitude of the association between the HLA gene type and the occurrence of CC or HPV infection was measured by OR and the respective 95% CI. Logistic regression analysis was carried out to examine the independent effects of multiple alleles found to be associated with disease. A linear trend was also analyzed using the 2 test when a trend was observed in the association between HLA and disease severity. The correlation between the alleles found to be associated with disease was computed using the Pearson correlation coefficient. The RR, as estimated by the odds ratio, was the measure used to determine the magnitude of the association between HLA and disease, and 95% CIs were calculated to determine the statistical significance of these associations.

Results

To verify whether the specific HLA-DRB1, B3, B4, and B5 alleles were associated with the risk of CC, the ORs and 95% CIs (Table 1) were calculated in 69 CC cases and 201 controls. The proportions of samples carrying HLA-DRB alleles are shown in Table 1. Two class II alleles that were present in > 20% of the cases, HLA-DRB1*13 (OR = 4.01 95% CI, 1.703 - 9.442 $p = 0.001$) and HLA-DRB1*3(17) (OR = 2.661 95% CI, 1.267-5.558 $p = 0.008$) were significantly associated with an elevated risk of CC. HLA-DRB1*13 alleles were analyzed including DRB1*13-1, DRB1*13-2 and other DRB1*13 alleles, but only DRB1*13-2 showed a strong association with an increased risk of CC. The other two class II alleles that were found to confer a decreased risk of CC were HLA-DRB1*09012 (OR = 0.182, 95% CI, 0.079 - 0.418 $p = 0$ and HLA-DRB1*1201 (OR = 0.35 95% CI, 0.142 - 0.863 $p = 0.018$), which were present in 38.31% and 21.39% of controls, respectively. The ORs also remained similar and marginally significant even after adjustment for all covariates.

Of the 201 control subjects included in the analysis, 101 harbored HPV DNA. The authors further examined the association between HLA alleles and HPV infection (Table 2). The OR for the HLA-DRB1*09012 (OR = 1.924; 95% CI, 1.08 - 3.427) and DRB3(52)*0101 (OR = 7.527 95% CI, 0.909 - 62.347 alleles increased in magnitude, and the risk associations of HPV infection for women carrying the two alleles would be increased. No significant negative associations were found in this analysis.

To investigate whether these associations were due to HPV infection, HPV type or to the development of cancer, the analysis was restricted to HPV16 positive control

subjects (n = 100) and HPV-16 positive case subjects (n = 40). HPV DNA was detected in 77% of the 69 tumors tested, and HPV16 was the most frequent oncogenic HPV type in the cases (75.5% of all cases). Firstly, the association between HLA alleles and CC risk was examined on the basis of HPV oncogenic potential, and the ORs for HLA-DR alleles were calculated considering only HPV-positive cancer cases in comparison with HPV positive control subjects (n = 100) (Table 3). A higher proportion of HPV16-positive samples carried the DRB1*15, DRB3 (52), and DRB1*08 alleles compared with control samples (with HPV infection). At the allele-specific level, significant positive associations were found for DRB3 (52)*02/03 (OR = 12.794; 95% CI, 5.007 - 32.691) and DRB1*3(17) (OR = 3.48; 95% CI, 1.261 - 9.604), although a risk trend was also observed for DRB1*13. On the other hand, two alleles, DRB1*09012 (OR = 0.122; 95% CI, 0.045 - 0.333) and DRB5 (51)*01/02 (OR = 0.222; 95% CI, 0.108 - 0.458), showed significant negative associations with the risk of CC in this analysis. Secondly, the risk estimates for specific alleles associated with CC were totally different when the case group was restricted to HPV-16-positive case subjects (n= 40), compared with HPV-16-negative case subjects (n = 29). The risks of CC infected with HPV-16 were statistically significantly different from those of HPV-16-negative cases, and two positive association DRB1*1501 (OR = 4.173; 95% CI, 1.065 - 16.356) and DRB5(51)*0101/0201 (OR = 4.173; 95% CI, 1.065 - 16.356) and a negative association with DRB4(53)*0101 (OR = 0.329; 95% CI, 0.122 - 0.888) were found (Table 4).

Discussion

In this population-based study, the authors analyzed the proportions of HLA class II DRB polymorphisms in 69 cases with CC and 201 control women from a high incidence area in the mid-west of China. The association between HLA class II alleles and increased or decreased risks of CC has been previously reported. The present results supported the hypothesis that certain HLA class II DRB alleles affect the risk of invasive CC. In this study, there were four HLA class II alleles significantly associated with CC after correction for multiple comparisons. The presence of the HLA DRB1*09012 and DRB1*1201 alleles was associated with a decreased risk of CC, and the strongest negative association was between DRB1*09012 and CC.

In the present study, the authors detected certain associations, that to their knowledge have not been reported previously, such as an increased risk of CC associated with HLA DRB3(17) and decreased risks associated with the DRB1*09012 and DRB1*1201 alleles. A significant positive association was found between the HLA DRB1*13 group and HLA DRB3(17) and an increased risk for CC. The strongest associations involved HLA DRB1*13-2, while other DRB1*13 alleles, although present, tended to be less frequent among CC cases. A decreased risk of cervical neoplasia associated with the HLA DRB1*13 has been previously reported [7, 8]. Although the DRB1*13 allele has been associated with a decreased risk of CC in several population studies throughout the world [8], the authors found positive associations between CC and the DRB1*13 allelic group. The strongest associations involved DRB1*13-2, which was associated with a four-fold increased risk of CC (OR = 11.5; 95% CI, 3.5 - 38.5 in Table 1). The reason for these conflicting findings between different studies is not clear, but it is possible that a direct association between DRB1*13 and cervical disease is not always present, and differences between the ethnically distinct populations in each study or the presence of diverse HPV types in different regions could be responsible for the discrepancies. These studies, which showed a positive association, were conducted among Mongolian populations in China. Although the associations detected in different populations frequently involve the same HLA groups, there is no consensus regarding the specific HLA alleles that contribute to the risks of CC in any particular population. Contradictory results could be an indication that such populations have intrinsic features that are determinants of risk, and suggest the possible role of differences in the interaction between environmental and host factors in the risk of CC.

It is likely that only women persistently infected with oncogenic HPV types are prone to developing malignant cervical lesions. Furthermore, persistent HPV infections associated with a high viral load are considered to be risk factors for the development of cervical lesions [9]. The outcomes of HPV infection are the result of a combination of features intrinsic to some HPV types (particularly those of the high-risk types) and interactions between HPV and the host [10, 11]. The authors investigated the associations between HLA class II alleles and HPV infection in a control population from the Wufeng region, which has one of the highest rates of incidence of CC and HPV infection in China. Significant associations were found between the HLA DRB1 *09012 and DRB3(52)*0101 alleles and an increased risk for HPV infection. The DRB3(52)*0101 allele was associated with a seven-fold increased risk of HPV infection (Table 2), showing the strongest relationship in the study. There were no alleles showing negative associations in the present study. However, the analysis was based on HPV measurements obtained at a single point in time, and therefore the interactive relationship between HLA polymorphisms, HPV infection persistence, and development of malignant lesions cannot be confirmed. The fact that DRB3(52)*0101 and DRB1*09012 were associated with increased risks for HPV positivity in this population may indicate the importance of HLA polymorphisms in directing the course of HPV infections. Genetic susceptibility may influence the persistence of HPV infections and high viral loads, augmenting the risk for development of malignant lesions of the cervix.

In addition, there was a nearly 13-fold increase in the risk of CC associated with the DRB3(52)*02/03 allele in this study (Table 3). The prevalence of this polymorphism

in China suggests that the presence of this allele could be considered a risk for the development of malignant lesions of the cervix in cases of oncogenic HPV16 infection. DRB1*3(17) had similar effects on the development of CC. DRB1*09012 (OR = 0.122; 95% CI, 0.045 - 0.333) and DRB5(51)*01/02 (OR = 0.222; 95% CI, 0.108 - 0.458) showed significant negative associations in this analysis, which have not been reported previously. However, the presence of the DRB1*09012 allele was associated with decreased risks for CC in the absence of HPV16 infection, but it was associated with increased HPV positivity among the control population. This indicates that the DRB1*09012 allele may be a significant HLA allele in carcinogenesis of the cervix regardless of the HPV infection status. Taken together, these findings suggest that different HLA II alleles may contribute differently to the development of CC and HPV infection in our population.

To better understand the mechanism by which HLA class II alleles affect the development of CC in the presence or absence of HPV infection, HPV-16 positive cases were compared to HPV16 negative cases and the results are shown in Table 4. The authors identified specific HLA alleles that increased or decreased the risk of cervical disease. The risks for CC in cases on oncogenic HPV were different (although not significantly) from those of non-HPV-16-containing tumors in association with DRB1*1501 and DRB5 (51)*0101/0201, which were associated with at least a four-fold increased risk of CC in the presence of HPV16 infection. These results indicated that these two alleles may play important roles in the development of CC induced by HPV16. DRB4(53)*0101 displayed a negative association with the risk of CC, and the presence of this allele prevented the development of CC in cases of persistent oncogenic HPV infection. However, it should be noted that DRB5(51)*0101/0201 decreased the risk of HPV containing cervical cancer (OR = 0.222, 95% CI: 0.108 - 0.458). These results suggest that, regardless of HPV type, there might be a decreased risk of cervical cancer associated with DRB5(51)*0101/0201, whereas a strong positive association is detected in the presence of HPV16 infection in cervical cancer tissues.

Although HPV infection is considered a major risk factor for the development of CC, it is not the only causative factor. There is increasing evidence of the essential role of cellular immune responses in HPV infection clearance and the development of CC [12-14]. The identification of an increasing number of risk associations for combinations of MHC genes may help explain some of the discrepancies regarding HLA-related susceptibility among individuals. Studies addressing the relationship between HLA and HPV-16 have found an increased risk of CC that contain certain HPV types associated with special DRB1 alleles [15-18]. Viral factors, such as type and variant, and viral load may promote cervical carcinogenesis in the context of the host's HLA type. The small sample size of the present study may have limited the detection of significant differences between HPV16 containing and non-HPV16 containing tumors . In addition, the HLA alleles may directly affect other tumor-associated antigens, rather than HPV peptides, or the relevant epitopes could be conserved across HPV types. Nevertheless, the possibility that these alleles play an important role in HPV infection susceptibility cannot be discounted.

Previous studies suggested that the risk of CC may depend on specific HLA alleles or HLA-linked genes. In addition, studies have indicated that the cellular immune response is essential in HPV infection clearance [12, 13]. Different combinations of HLA class II molecules and antigenic peptides may influence cytokine production during the early stages of the immune response against an HPV infection [19, 20]. As genotyping of the HLA region has become more precise, observations of associations between HLA and CC have also become more refined. A decreased susceptibility to cervical dysplasia was associated with the haplotype DRB1*0101 in British women [21]. Several studies have examined the association between the risks of cervical neoplasia and the presence of these alleles [7, 22, 23]. Some recent studies conducted in the eastern United States and Costa Rica with high-resolution typing across A-B-DR alleles reported that there were no HLA haplotypes significantly associated with cervical neoplasia [24]. However, less consistent results for different HLA alleles similar to those of the present study have been previously reported and may represent population-specific or even chance findings because of the polymorphic nature of the HLA region genes. Although different populations frequently exhibit associations involving the same HLA alleles, there is no consensus regarding the specific HLA alleles that contribute to the risks of CC or HPV infection in any particular population [7, 19-22].

In conclusion, the present results suggest that HLA polymorphisms play a role in the genetic susceptibility to CC and HPV infection in the Chinese population. In particular, the authors found an increased risk for CC associated with the HLA DRB1*13-2, HLA DRB3(17) and DRB3(52)*0201/03 alleles. Individuals that carry the HLA DRB1*09012, DRB1*1201 and DRB4(53)*0101 alleles were found to have decreased risks for CC. An increased risk for HPV positivity was associated with DRB3(52)*0101. A better understanding of these associations may help clarify the role of HLA molecules in the immune responses against HPV infections and subsequent CC pathogenesis in the Chinese population, and would be of value for the design of strategies for the prevention and treatment of CC through vaccination and immunotherapy.

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Effects of volume-activated chloride channels on the invasion and migration of human endometrial cancer cells

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Summary

Objective: To investigate the role of volume-activated chloride channels (VACC) in invasion and migration of human endometrial cancer cell. *Materials and Methods:* Expression of voltage-gated chloride channel-3 (CLC-3) was detected by employing reverse transcriptase-polymerase chain reaction (RT-PCR) in human endometrial cancer Ishikawa cell line. Cell invasion and cell migration were determined by using the Transwell invasion and migration assay, respectively. NPPB, a Cl^- channel blocker, was treated to observe the effects of volume-activated Cl^- channel on invasion and migration of endometrial cancer cell. *Results:* CLC-3 RNA expression was observed in Ishikawa cell line. The authors showed that blockade of Cl^- channels specifically inhibited invasion and migration of endometrial cancer Ishikawa cell line in a dose-dependent manner. VACC activation and subsequent regulatory volume decrease (RVD) were markedly suppressed by NPPB. Anion replacement studies indicate that permeation of Cl^- ions through endometrial cancer Cl^- channel is obligatory for regulatory volume decrease (RVD) induced by VACC. Moreover, $[Ca^{2+}]_i$ measurements indicated that VACC-mediated increase in $[Ca^{2+}]_i$ was one of the mechanisms of cancer cell invasion and migration. *Conclusions:* These data intensely suggest that VACC in endometrial cancer may facilitate tumor invasion and migration, presumably through inducing RVD and mediating $[Ca^{2+}]_i$ increase.

Key words: Chloride channels; Invasion; Migration; Endometrial cancer.

Introduction

Chloride channels are ubiquitous transmember proteins involved in diverse cellular processes. They are essential for salt and fluid movements across epithelia and volume regulation. According to gating mechanisms, there are from a functional point of view, five classes of chloride channels, including volume-activated chloride channel (VACC) (also named as swelling-activated chloride channel and volume-sensitive or -regulated chloride/anion channel). An increase in cell volume evokes a series of signaling events resulting in activation of volume-sensitive chloride channel. The effluxes of Cl^- ions through the Cl^- channel followed by water lead to a cell volume decrease referred to as regulatory volume decrease (RVD). There is a continuing effort to identify the molecular structure of the VACC. Although CLC-3, an attractive voltage-gated chloride channel (CLC) gene family member, might be involved in the activation or modulation of volume-activated chloride current, the molecular nature of VACC is still controversial. Nevertheless, CLC-3 has been considered the most likely molecular candidate of VACC [1, 2]. Despite difficulties concerning the molecular identification of VACC, there is a good agreement on the properties which include blockade by 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) [3, 4]. The activation of VACC by volume changes and its influence on cell volume make it a prime candidate for participation in the shape-volume changes.

In a previous study [5, 6], the authors found that the chloride channel is correlated with the malignant biology manner of ovarian cancer cell. However, there are no data available as to whether the VACC affects invasion and migration of endometrial cancer. The authors hypothesize that the activation of VACC, with its accompanying water efflux, results in cell shrinkage (RVD) and increase of intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) that are a requirement for successful invasion of endometrial cancer cells. Hence, VACC may aid the invasive biology of endometrial cancer cells, a feature that greatly compromises surgical treatment of this disease.

Materials and Methods

Cell culture

Human endometrial cancer Ishikawa cell line was obtained from Basic Medicine Research Institute, Qilu Hospital, Shandong University (China). Cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and maintained at 37°C in a humid atmosphere of 5% CO_2 in air.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of CLC-3 expression

Total RNA was isolated from the cultured Ishikawa cell line using the Trizol reagent according to the manufacture's procedure; mRNA was transcribed into first strand cDNA using oligo-dT primers and M-MLV reverse transcriptase. The sequences used were: CLC-3 primer, sense, 5'GGCAGCATTAACAGTTCTACAC3'; antisense, 5'TTCCAGAGCCACAGGCATATGG3'. β-actin primer, sense, 5'AACTCCATCATGAAGTGTGA3'; antisense, 5'ACTCCTGCTTGCTGATCCAC3'. PCR cycling conditions were as follows: an initial denaturation step of 94°C for 5 min, 94°C for 1 min, annealing at 58°C for 1 min, and elon-

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gation at 72°C for 1 min. A final elongation step of 10 min at 72°C occurred on the last cycle. PCR reactions were cycled 40 times. Control reactions without reverse transcriptase were performed for each PCR amplification experiment. The PCR products were analyzed by electrophoresis in a 2% agarose gel and stained with ethidium bromide (0.5 μg/ml).

Invasion and migration assays

The invasiveness of endometrial Ishikawa cancer cell was assayed using Transwell chambers. Briefly, polycarbonate filter (pore size, 8 μm) was coated with 100 μl of Matrigel. Serum-free RPMI-1640 containing 1.0×10^5 cells in 100 μl was introduced into the upper compartment and conditioned medium used as chemoattractant was added into the lower chamber. NPPB were added to both upper (with cells) and lower chambers at the desired concentrations. The cells were allowed to invade the Matrigel for 24h at 37°C in a 5% CO_2 atmosphere. Cells that had penetrated the Matrigel were quantified by MTT assay. Cells on the upper surface of the filter that had not invaded through the Matrigel were removed with a cotton swab. Cells that had invaded, remained on the filter and 25 μl MTT solution (5 mg/ml) was added to each well. After four hours of incubation at 37°C, the cells on the filter formed dark-blue crystals. The filter was then placed into another well containing 150 μl of DMSO to dissolve the crystals. After 30 min, the solution was poured into 96-well plates and absorbance was measured. To assess cellular migration potential, the protocol described above was used, except that Matrigel was omitted.

Cell volume measurements and ion replacement assay

Ishikawa cells were perfused with saline solution consisting of (in mM): NaCl, 122.6; KCl, 5.0; $MgCl_2$, 1.2; $CaCl_2$, 2.0; $Na2HPO_4$, 1.6; NaH_2PO_4, 0.4; glucose, 10.5; HEPES 5.0; $NaHCO_3$, 25.0; and Na_2SO_4, 1.2, pH 7.4. For osmotic challenges, this solution was modified by removing 50 mM NaCl in the case of hypo-osmotic solution (200 mOsm/kg) and adding back mannitol for the isotonic (308 mOsm/kg) solution. In this manner, solutions were maintained isoionic. During the ion replacement experiment, cells were superfused with a solution containing (in mM): Na gluconate 130, K gluconate 5.4, $MgSO_4$ 0.8, Ca gluconate 1.2, NaH_2PO_4 1, glucose 5.5, and Tris 5, pH-adjusted to 7.4. To obtain a hypotonic solution, the sodium gluconate was reduced to 80 mM. Then the coverslips were mounted on an inverted microscope with a CCD camera. Cell images were captured every 30 sec. throughout the entire experiments and were then analyzed with an image analysis software. Cell volume was calculated using the equations of $V = 4/3 \times S \times (S/\pi)^{1/2}$, where S is the area ($\mu m^2$). RVD was calculated with the equation of $RVD\ (\%) = (V_{max}\text{-}V_{min}) \div (V_{max}\text{-}V_0) \times 100\%$, where V0 is the cell volume in isotonic solution before hypotonic stimulation, V_{max} is the peak volume in hypotonic solution, Vmin is the volume before return to isotonic solution. Drugs were added to the isotonic and hypotonic solutions to final desired concentrations of NPPB. All experiments were performed at room temperature (22°C - 26°C).

$[Ca^{2+}]_i$ measurements

Ishikawa cells were grown overnight in 90% RPMI-1640 supplemented with 10% FBS containing 100 μU/ml penicillin and 100 μg/ml streptomycin on circular discs at 37°C and 5% CO_2 in air. Cells were loaded with Fura-2/AM for 1 hr in the dark at room temperature by incubation with 10 μM membrane-permeant Fura-2/AM in a physiological solution that contained (in mM): NaCl, 122.6; KCl, 5.0; $MgCl_2$, 1.2; $CaCl_2$, 2.0; Na_2HPO_4, 1.6; NaH_2PO_4, 0.4; glucose, 10.5; HEPES 5.0; $NaHCO_3$, 25.0; and Na_2SO_4, 1.2, pH 7.4. For osmotic challenges, this solution was modified by removing 50 mM NaCl in the case of hypo-osmotic solution (200 mOsm/kg) and adding back mannitol for the isotonic (308 mOsm/kg) solution. In this manner, solutions were maintained isoionic. Drugs were added to the hypotonic solutions to final concentrations of NPPB. Ca^{2+} influx was measured by the changes in fluorescent signals as recorded by a LS-50B luminescent spectrometer at excitation wavelength of 340/380 nm and emission wavelength of 510 nm. The ratio of the two images 340/380 was calculated and converted to absolute $[Ca^{2+}]_i$ concentrations.

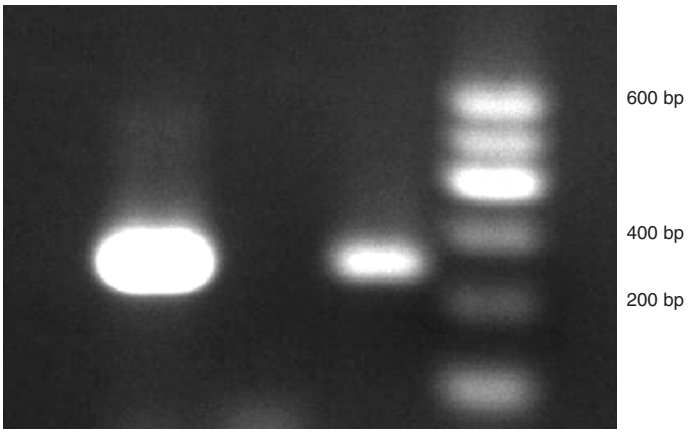

Figure 1. — RT-PCR analysis of the expression of CLC-3 transcript in human endometrial cancer Ishikawa line.

Statistics

Data were presented as the mean ± standard error. Student's t-test was used for statistical analyses and differences were considered significant at $p < 0.05$. All experiments were performed at least three times with representative data presented.

Results

RT-PCR assay

Unfortunately, the molecular structure of VACC is not known. After many candidates, only CLC-3, an attractive member of CLC family, is still considered as being potentially involved in volume-regulated chloride currents. Thus, in trying to find a possible correlation of VACC with biological behavior of endometrial cancer, from the molecular basis, the authors' strategy was firstly to determine whether CLC-3 is expressed in the Ishikawa cell line. Total RNA was extracted and RT-PCR analysis was performed using primers specific for CLC-3 and β-actin. Figure 1 shows that PCR notably amplified a 235-bp CLC-3 and 247-bp β-actin from total RNA isolated from Ishikawa cells. No product was detected in the absence of reverse transcriptase.

Fig. 2

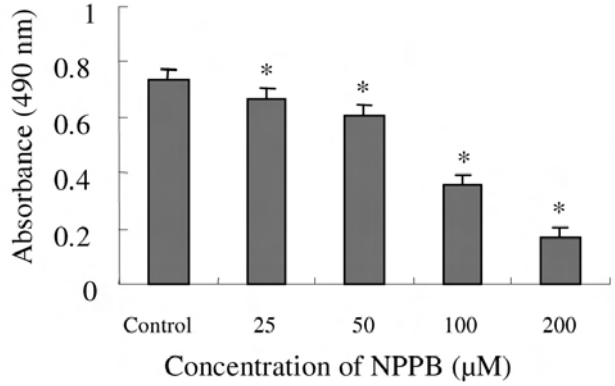

Fig. 3

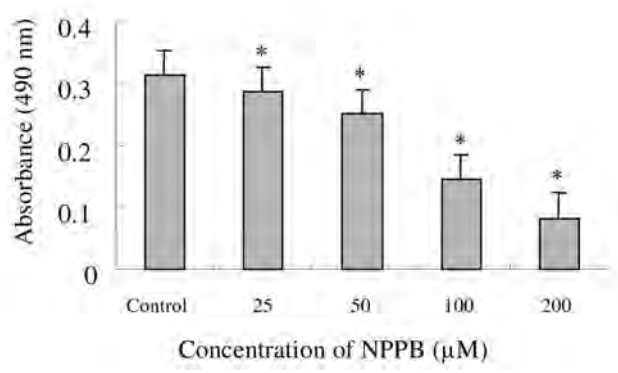

Fig. 4

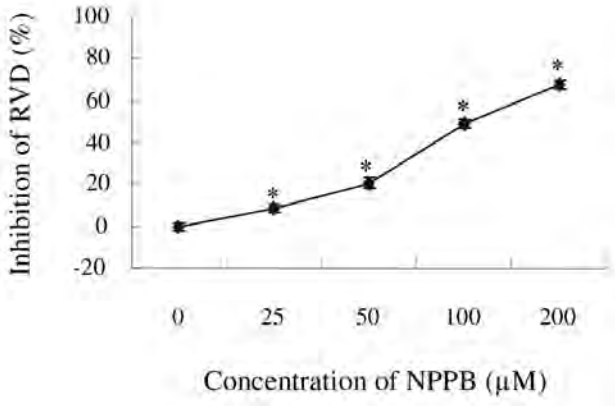

Fig. 5

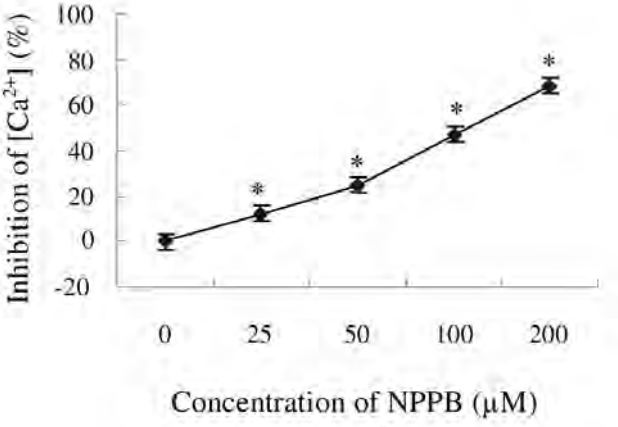

Figure 2. — Inhibition of Ishikawa cell invasion by NPPB. Cells were cultured in medium without (control) or with desired concentrations of chloride channel blocker NPPB. Ability of cell invasion was determined using Transwell chamber. Absorbance value, which indicated relative cell number, was detected employing the MTT method. * $p < 0.05$ compared with control.

Figure 3. — Inhibition of Ishikawa cell migration by NPPB. Cells were cultured in medium without (control) or with desired concentrations of chloride channel blocker NPPB. Ability of cell migration was determined using Transwell chamber. Absorbance value, which indicated relative cell number, was detected employing the MTT method. * $p < 0.05$ compared with control.

Figure 4. — Inhibition Ishikawa cell RVD by NPPB. Cells were perfused in hypotonic solution without (control) or with desired concentrations of chloride channel blocker NPPB. It was shown using cell volume measurement that NPPB inhibited Ishikawa cell RVD induced by hypotonic stimulation in a dose-dependent manner. *$p < 0.05$ compared with control.

Figure 5. — Inhibition Ishikawa cell $[Ca^{2+}]_i$ increase by NPPB. Cells were perfused in hypotonic solution without (control) or with desired concentrations of chloride channel blocker NPPB. It was shown by $[Ca^{2+}]_i$ measurement that NPPB inhibited Ishikawa cell $[Ca^{2+}]_i$ induced by VACC in a dose-dependent manner. *$p < 0.05$ compared with control.

Effects of Cl^- channel blocker on Ishikawa cell invasion and migration in vitro

To investigate the roles that VACC plays in the invasive behavior of endometrial cancer, the authors used a Transwell migration assay frequently used to assess cell chemotaxis and invasiveness. Ishikawa cells were plated on the upper side of a filter insert containing eight μm pores and were attracted to migrate through these pores towards the Matrigel. After 24 hrs incubation, the number of invaded cells was quantified using MTT assay. Invasion of Ishikawa cells was greatly reduced by 9.2 ± 1.3% ($p < 0.05$), 17.3 ± 3.1% ($p < 0.05$), 51.8 ± 3.9% ($p < 0.05$), 77.1 ± 4.2% ($p < 0.05$) in the presence of 25 μm, 50 μm, 100 μm, and 200 μm NPPB (Figure 2). After six hrs incubation, migration of Ishikawa cells was significantly inhibited by 8.6 ± 1.5% ($p < 0.05$), 20.3 ± 2.8% ($p < 0.05$), 53.6 ± 3.5% ($p < 0.05$), and 73.7 ± 3.6% ($p < 0.05$) in the presence of 25 μm, 50 μm, 100 μm, and 200 μm NPPB, respectively (Figure 3).

The effect of Cl^- channel inhibitor on RVD

Cell volume adaptive changes are critical for any cell survival. To investigate whether inhibition of Cl^- channel by the drug treated for invasion and migration assays perturb the tumor cells, ability to regulate cell volume, the authors experimentally altered the cell volume of Ishikawa cells by changes in bath osmolality and observed the effects of Cl^- channel inhibitor on volume regulation. They also rendered Ishikawa cells to a brief hypo-osmotic swelling and monitored their gradual volume decrease towards the original cell size. Data demon-

strated that exposure of cultured Ishikawa cells to a hypotonic solution (200 mOsm) induced rapid cell swelling, which was followed by a slow recovery up to 80% of the initial volume. Subsequently, the authors carried out an ion replacement experiment to prove Cl^- role in the RVD. Cells were perfused with a gluconate-based isotonic chloride-free solution for three hrs to deplete the intracellular Cl^-, and then perfused with a gluconate-based hypotonic chloride-free solution. Results indicated that RVD was almost completely abolished. However, cells recovered RVD when gluconate-based hypotonic solution was replaced with chloride-based hypotonic solution. Pretreatment with NPPB, which was added to the hypotonic solution, resulted in inhibition of RVD in a dose-dependent manner (Figure 4). NPPB at 25, 50, 100, and 200 μm inhibited RVD by 8.7 ± 2.1% ($p < 0.05$), 20.8 ± 2.3% ($p < 0.01$), 49.2 ± 3.7% ($p < 0.01$), and 67.4 ± 3.5% ($p < 0.01$), respectively. Taken together, these experiments showed that Cl^- plays a key role in cell swelling-mediated RVD; and NPPB, a conventional chloride channel blocker, can inhibit cell swelling-mediated RVD in a dose-dependent response.

The effect of Cl^- channel inhibitor on $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ is a important parameter in modulating actin filament turnover, and increase of $[Ca^{2+}]_i$ is part of the regulatory volume decrease. To further explore the mechanisms of VACC involvement in invasion and migration of endometrial cancer, the authors examined the effect of Cl^- channel inhibitor on Ca^{2+} influx. Exposure of cultured endometrial Ishikawa cancer cell to a hypotonic solution induced rapid cell swelling. This was followed by a slow recovery within the next 15 min, also referred to as RVD. Interestingly, the application of hypotonic media elicited a sharp rise of $[Ca^{2+}]_i$ to a peak concentration with 600 nM above its levels in isotonic conditions. Pretreatment with NPPB which was also added in the hypotonic solution, significantly reduced the peak Ca^{2+} response in a dose-dependent manner (Figure 5). NPPB at 25, 50, 100, and 200 μm inhibited the peak Ca^{2+} response by 12.1 ± 2.8% ($p < 0.01$), 24.6 ± 3.4% ($p < 0.01$), 47.0 ± 3.1% ($p < 0.01$), and 68.1 ± 4.5% ($p < 0.01$), respectively.

Discussion

There is ample evidence that indicates an important role for volume-activated chloride currents in regulating the proliferation and multidrug resistance of tumor cells [7, 8]. Currently, little information is known about its effect on malignant biology behavior of endometrial cancer cells. Although the molecular nature of VACC is not determined, CLC-3 which is a member of the voltage-gated CLC, has been considered the most likely molecular candidate of the VACC. The data in this present study showed that endometrial cancer Ishikawa cell line notably expressed CLC-3 by RT-PCR assay. Moreover some studies demonstrated that VACC contribute to the migration and invasion of cancer cells [9, 10]. In the present study, treatment with NPPB significantly inhibited invasion and migration of endometrial cancer cells in a dose-dependent response. At the same time, the authors found that Cl^- was necessary for RVD by ion replacement experiment and VACC-dependent RVD was positively correlated with invasion and migration of endometrial cancer cells. It is implicated that the VACC may play an important role in the invasion and migration of endometrial cancer cells.

How VACC are involved in control or regulation of cell invasion and migration is at present not clearly understood. VACC activation results in Cl^- efflux that is accompanied by cation and water efflux causing cell RVD. Cytoskeletal reorganization which is related to cell motility and migration is markedly affected by cell volume changes. In many cell types, RVD is associated with an increase, and cell swelling with a decrease in F-actin content [11]. Volume-sensitive Cl^- channels are also proposed to be related to the cytoskeleton or motility of the cells [12]. The authors, therefore speculate that it is possible that the activation of VACC and subsequent RVD may confer the endometrial cancer cells to a selective advantage for invasion and migration by cytoskeletal mechanisms. In fact, this conclusion is supported by the authors' studies in which VACC-dependent RVD is positively correlated with invasion and migration of endometrial cancer cells. Moreover, ion replacement experiment and endometrial cancer cell volume showed that Cl^- channel blocker (NPPB) reduced transmembrane Cl^- fluxes and greatly reduced osmotically-induced cell volume changes.

Ca^{2+} is an intracellular second messenger that plays a central role in signal transduction for many cellular responses. Many reports suggest that exposure of cells to hypoosmotic solutions has been shown to increase intracellular Ca^{2+} levels [13, 14]. RVD in epithelial cells is thought to be dependent on a rise in $[Ca^{2+}]_i$ [15]; moreover the distribution of intracellular free Ca^{2+} is not uniform. The rise of $[Ca^{2+}]_i$ which is more prominent in the cell body than in the lamellipodium [16] can loosen cell-matrix connections at the rear end [17] and isolate the cortical actomyosin gel, thus allowing a contraction at the rear end [18]. Thus, VACC-mediated volume loss and $[Ca^{2+}]_i$ increment mechanisms may act in concert during retraction of the rear end of a migrating cell. In this study, hypotonic stimulation caused a sharp rise of $[Ca^{2+}]_i$ in endometrial cancer cells. Interestingly, NPPB significantly inhibited the increase of $[Ca^{2+}]_i$ evoked by external Ca^{2+} in a dose-dependent manner, indicating that VACC may modulate Ca^{2+} influx into endometrial cancer cells, and subsequently regulate the invasion and migration of these cells by the cytoskeletal mechanisms. Considering the present study, the authors would like to present a network model to interpret how VACC affects invasion and migration of cancer cells. VACC activation results in RVD which affects the cytoskeletal reorganization, probably contributing to the invasion and migration of endometrial cancer. Meanwhile VACC activation gives rise to $[Ca^{2+}]_i$ increase which mediates RVD, loses cell-matrix connections, and is linked to cytoskeletal depolymerization and polymerization. A final result is that cancer cells develop

more invasively. In contrast, VACC blockers could interrupt these pathways, therefore inhibiting the invasion and migration of Ishikawa cancer cell. As to how the VACC affects Ca^{2+} influx, as well as what the link is between RVD and $[Ca^{2+}]_i$ in endometrial cancer, the authors are ready to investigate these in future work.

In summary, the present findings indicate that VACC may be essential for invasion and migration of endometrial cancer cell. It is likely that they modulate invasion and migration through RVD and $[Ca^{2+}]_i$ changes. Therefore, targeting VACC may provide a novel way to inhibit invasion and migration within tissues. It could be worthwhile to further explore the possibility of employing Cl^- channel blockers as a new class of antineoplastic drugs.

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Predictive factors of malignancy in patients with adnexal masses

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Summary

Introduction: Good preoperative tumor triage is essential for choosing the appropriate approach. *Objective:* The study aim was to identify factors from standard preoperatively collected data, which could predict the nature of adnexal masses prior surgery. *Material and Methods:* The study involved all women treated in the Clinic for Gynecology and Obstetrics Clinical Center of Serbia for adnexal tumors throughout a period of 18 months. On admission, detailed anamnestical and laboratory data were obtained and ultrasound scans were performed. Obtained data were compared with hystopathological findings of tumors. Methods of correlation and logistic regression were applied to create association models. *Results:* Three new models for predicting tumor nature were achieved from anamnestical data, characteristics of women and tumors, and laboratory analyses. Two statistically significant (p = 0.000) equations were obtained for anamnestical data and characteristics of women and tumors, while three were made for laboratory analyses. Sensitivity of anamnestical malignancy index (AMI) was 73.33%, specificity 72.87%, positive predictive value (PPV) 39.49% and negative predictive value (NPV) 91.88%. Sensitivity of characteristic malignancy index (CMI) was 92.38%, specificity 67.36%, PPV 40.59% and NPV 97.34%. Sensitivity of laboratory malignancy index (LMI) was 56.45%, specificity 90.24%, PPV 68.63%, and NPV 84.57%. *Conclusions:* The best predictors of malignancy are menopausal status, body mass index (BMI), age, metastases, ascites, tumor marker CEA level, and erythrocyte sedimentation rate (ESR). Along with the risk of malignancy index (RMI), for more reliable triage and preoperative tumor evaluation the authors propose introduction of another three indexes (AMI, CMI, LMI) in clinical practice.

Key words: Adnexal masses; Preoperative triage; Predictors; Models.

Introduction

Although ovarian cancer, in the form of a malignant epithelial tumor, represents only the seventh most common malignant tumor in women, it is the fourth most common cause of death [1]. Good preoperative discrimination between benign and malignant ovarian tumors results in more women being appropriately referred for gynecologic oncology care and more women with benign conditions undergoing conservative surgical treatment [2]. The risk of malignancy index (RMI) is so far the best and most widely used tool for preoperative identification of patients with ovarian cancer [3]. However, currently there is no effective tool available to reliably predict the nature of adnexal masses and there are still significant false results [4]. Therefore, further research on recognition of new and trustworthy parameters that can preoperatively assess tumor nature is needed. The aim of this study was to identify factors from standard preoperatively collected data which could predict the nature of the adnexal masses prior to surgery.

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Materials and Methods

The study included all consecutive patients operated for adnexal tumors at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia throughout the period of 18 months (January 1, 2010 to June 30, 2011). All variables used in the protocol of the Clinic upon admission for adnexal tumor operation were prospectively collected for every patient and considered for association with the histopathological (HP) finding. Clinical and ultrasound informations were collected in a standardized manner. Clinical information obtained anamnestically regarded patient demographics (age, educational level, occupation, and residency) and gynecological data (family and personal history of gynecological and other diseases, menarche age, parity, last menstrual cycle, symptoms). Standard laboratory analysis included erythrocyte sedimentation rate (ESR), tumor marker levels (CA-125, CEA, Ca 19.9, Ca 15.3). Furthermore, body mass index (BMI) was calculated using the regular formula BMI = body weight (kg)/height^2 (m^2). Aside from clinical examinations of pelvic organs, a detailed/expert ultrasonographic scan was performed. The authors used HDI 5000, Sono CT, and Xres, with endovaginal-V8-4MHz (V) probe and its associated software. Ultrasound scan was performed by two experienced doctors. Risk of malignancy index (RMI) was calculated using the proposed formula: RMI = U x M x CA-125 [5]. In the formula, U represents the ultrasonographic index. In multilocular and bilateral tumors, the presence of solid areas in tumor, metastasis and ascites are marked with one point each. The sum of these points, are scored so that U 0 = 0 points, U 1 = 1 point, U 2-5 = 3 points. In the formula, M represents

menopausal status (1 for premenopausal and 3 for postmenopausal women). Levels of CA-125 are calculated directly to the equation. RMI < 25 shows low risk, 25-250 intermediate risk, while RMI > 250 shows high risk for malignancy. When different masses were present, only information from the most complex one was used for analysis. Standard operative procedures appropriate for the staging of the tumor were undertaken and all tumors were extracted and sent for HP analysis. Postoperatively, final HP enabled to classify each mass as benign, borderline, or malignant. First simple correlations of examined parameters and HP findings, as well as RMI were done. Then, all examined variables were divided into three groups and evaluated their correlation with tumor type, for the whole group. The first group regarded anamnestical data (personal and family illness history, employment status, menopausal status menarche time, number of births, miscarriages, and abortions), the second one included characteristics of women (age, presence of symptoms, and BMI) and tumors (dimensions, multilocularity, and bilateralism, solid parts, metastases, and ascites), and the third consisted of laboratory analyses (tumor marker levels, RMI, and ESR). Having all the necessary information, models for preoperative malignancy predictions were made. The authors calculated numerical values in the examined population of these newly-constructed overall models: anamnestical malignancy index (AMI), characteristic malignancy index (CMI), and laboratory malignancy index (LMI). The parametrical factors were calculated directly into the equations. Non-parametrical factors were categorized and scored. Having positive family history of gynecological diseases or being in menopause received one point while the opposite situation was scored with two points. Ascites presence received one point and absence zero points. Tumors < five cm were scored with one point, from five to ten cm with two points, while > ten cm with three points. The cut-off point for each index was determined. Finally, sensitivity [(true positive/true positive + false negative) x 100], specificity [(true negative/true negative + false positive) x 100], positive [(true positive/true positive + false positive) x 100], and negative [(true negative/true negative + false negative) x 100] predictive values were calculated for AMI, CMI, and LMI.

In the statistical analysis, for the general description percentages (%) were mostly used. Spearman correlation was used to determine associations between tumor types or RMI and individual investigated parameters as well as for model values and HP findings. At the end, multivariate binary logistic regression was applied to investigate the relationships of groups of investigated parameters together and the tumor type (benignant/malignant) in order to form equations that could be of use in preoperative patient assessment. The level of significance was $p < 0.05$. Obtained data were analyzed using the SPSS software (Advanced Statistics, version 17.0, Chicago, IL).

Results

This study involved 520 women. HP revealed that 85 (15.74%) women had malignant while 435 (80.56%) had benignant tumors.

HP findings significantly correlated with patients' age ($p = 0.257$; $p = 0.000$), employment status ($p = 0.100$; $p = 0.020$), menopausal status ($p = 0.252$; $p = 0.000$), number of births women had ($p = 0.904$; $p = 0.029$), number of abortions ($p = 0.159$; $p = 0.000$), women's BMI ($p = 0.108$; $p = 0.000$), presence or absence of metastases ($p = 0.576$; $p = 0.000$), ascites ($p = 0.455$; $p = 0.000$), Ca 125 level ($p = 0.272$; $p = 0.000$), Ca 15.3 level ($p = 0.468$; $p = 0.000$), RMI ($p = 0.296$; $p = 0.000$) and ESR ($p = 0.279$; $p = 0.000$).

RMI significantly correlated with HP findings ($p = 0.296$; $p = 0.000$), menopausal status ($p = 0.364$; $p = 0.000$), number of miscarriages ($p = 0.142$; $p = 0.001$), BMI ($p = 0.137$; $p = 0.000$), symptoms ($p = 0.117$; $p = 0.007$), Ca 19.9 level ($p = 0.178$; $p = 0.004$), Ca 15.3 level ($p = 0.305$; $p = 0.001$), ESR ($p = 0.284$; $p = 0.000$) and patients age ($p = 0.246$; $p = 0.000$). As expected, the characteristics that are assessed in order to calculate RMI are all highly and significantly correlated with RMI values: multilocularity ($p = 0.217$; $p = 0.000$), solid parts ($p = 0.142$; $p = 0.001$), metastases ($p = 0.341$; $p = 0.000$), ascites ($p = 0.307$; $p = 0.000$), bilaterality ($p = 0.138$; $p = 0.001$) and Ca 125 level ($p = 0.801$; $p = 0.000$).

As numerous simple correlations were established and almost all examined parameters were associated either with tumor type HP or with the RMI, all variables were divided into three groups and evaluated their correlation with tumor type, for the whole group.

When anamnestical characteristics of examined women were assessed all together, a significant model - anamnestical malignancy index (AMI), was achieved in Enter method ($\chi^2 = 102.959$; $p = 0.000$). The model's total calcification success was 82.4% and R^2 Nagelkerke 0.277. Menopausal status and family history of gynecological illnesses were significant parameters. Therefore, using Forward Wald method, two equations were constructed ($\chi^2 = 73.466$; $p = 0.000$; R^2 Nagelkerke = 0.203 and $\chi^2 = 92.686$; $p = 0.000$; R^2 Nagelkerke = 0.252) (Table 1).

When characteristics of women and tumors were assessed together, a significant model - characteristic malignancy index (CMI), was achieved in Enter method ($\chi^2 = 273.425$; $p = 0.000$). The model's total calcification success was 91.3% and R^2 Nagelkerke 0.635. Patients' BMI and age, ultrasound scan estimated diameters, and presence of ascites were significant parameters (Table 1). Using the Forward Wald method, one more parameter – metastases, proved to be significant ($\chi^2 = 149.561$; $p = 0.000$; R^2 Nagelkerke = 0.386) (Table 1).

When findings of laboratory analyses were assessed together, a significant model - laboratory malignancy index (LMI) was achieved in Enter method ($\chi^2 = 48.868$; $p = 0.000$). The model's total classification success was 85.9% and R^2 Nagelkerke 0.671. No specific parameters were pointed out. However, using Forward Wald method, three equations were created ($\chi^2 = 31.913$; $p = 0.000$; R^2 Nagelkerke = 0.488; 2 = 37.439; $p = 0.000$; R^2 Nagelkerke = 0.533; 2 = 49.729; $p = 0.000$; R^2 Nagelkerke = 0.624) (Table 1).

Numerical values for the overall models achieved for three investigated factor groups (AMI, CMI, LMI) were calculated. Mean ± SD of AMI was 1.73 ± 1.06 (min = -1.55; max = 2.51). Mean ± SD of CMI was 2.85 ± 1.73 (min = -2.31; max = 5.82). Mean ± SD of LMI was 4.75 ± 12.18 (min = -9.52; max = 124.52). Obtained indices were correlated with HP findings and RMI as the most

Table 1. — *Logistic regression equations for preoperative malignancy calculation.*

Parameters	Models
Anamnestical data - AMI	Malignancy = 4.303 – 1.953 x menopausal status
	AMI: Malignancy = 0.352 + 2.069 x gynecological ilnesses in family – 1.984 x menopausal status
Characteristics of women and tumors - CMI	Malignancy = 1.914 + 23.117 x metastases
	CMI: Malignancy = 10.269 – 0.130 x BMI – 0.559 x US tumor dimensions – 2.669 x ascites – 0.057 x age
Laboratory analyses - LMI	Malignancy = 1.395 + 0.002 x RMI
	Malignancy = 2.633 + 0.002 x RMI – 0.025 x ESR
	LMI: malignancy = 3.168 - 0.259 x CEA + 0.002 x RMI – 0.031 x ESR

reliable parameter for prediction of tumor nature so far. All three indices were significantly correlated with HP (AMI $p = 0.280$; $p = 0.000$; CMI $p = 0.380$; $p = 0.000$; LMI $p = -0.195$; $p = 0.003$). AMI was HP significantly correlated ($p = 0.593$; $p = 0.000$) with CMI. AMI and LMI ($p = -0.044$; $p = 0.510$), as well as CMI and LMI were not significantly correlated ($p = -0.097$; $p = 0.145$). Moreover, all three new indices were also significantly correlated with RMI: AMI $p = -0.333$; $p = 0.000$; CMI $p = -0.311$; $p = 0.510$; LMI $p = -0.460$; $p = 0.510$.

Cut of values were set. AMI < 1 showed possible malignancy and ≥ 1 benignancy. CMI < 3 showed possible malignancy while ≥ 3 benignancy. LMI ≥ 3 showed possible malignancy and < 3 benignancy. Sensitivity of AMI was 73.33%, specificity 72.87%, positive pradictive value (PPV) 39.49%, and negative predictive value (NPV) 91.88%. Sensitivity of CMI was 92.38%, specificity 67.36%, PPV 40.59%, and NPV 97.34%. Sensitivity of LMI was 56.45%, specificity 90.24%, PPV 68.63%, and NPV 84.57%.

Discussion

Ovarian cancer has the worst prognosis among all forms of gynecological malignancies, mainly due to the lack of an effective screening method for the detection of early-stage disease [6]. An accurate preoperative diagnosis of adnexal masses is essential to provide optimal treatment [2]. Therefore, a number of strategies have been proposed to triage women with suspicious adnexal tumors [7]. Recently made multivariate logistic regression models have been introduced using a variable set of demographic, clinical, tumor marker, and ultrasound characteristics [8-11]. Over the past years, various screening methods and challenging biostatistical algorithms have been developed and validated in order to estimate the absolute risk of having ovarian cancer in women with and without symptoms [12]. Thus, it is becoming possible to analyze the relevance of combinations of markers for identification of tumor type [13]. Nevertheless, most logistic regression models were developed to discriminate benign from malignant tumors, using small sample sizes [2]. This study provides new insights into preoperative evaluation of adnexal masses using statistical modeling in a larger group of patients.

Some investigators state that in order to identify ovarian cancer with reasonable accuracy, the age of patients, their family history, serum level of CA-125, and ultrasound findings should always be taken into consideration simultaneously [11, 14]. Other studies found that the most useful independent prognostic variables for the logistic regression model were: personal history of ovarian cancer, hormonal therapy, age, maximum diameter of lesion, pain, ascites, blood flow within a solid papillary projection, presence of an entirely solid tumor, maximal diameter of solid component, irregular internal cyst walls, acoustic shadows, and a color score of intratumoral blood flow. Sensitivity of this model was 93% and specificity was 76% [15]. When researchers placed as risk factors age, family history of ovarian cancer, previous cancers other than ovarian, BMI, smoking, alcohol, deprivation, loss of appetite, weight loss, abdominal pain, abdominal distension, rectal bleeding, postmenopausal bleeding, urinary frequency, diarrhea, constipation, tiredness, and anaemia, a model explained 56% of variance [12]. The authors attempted to take into consideration all available parameters used in daily practice. The models involved similar parameters as the ones from literature and had comparable and satisfactory sensitivity and specificity.

Well-known risk factors for malignancy include: nulliparity, low parity, delayed childbearing, early onset of menses, late menopause, postmenopausal estrogen use for ten or more years, and a family history of ovarian or breast cancer [7, 14]. Furthermore, higher BMI was associated with an increased ovarian cancer risk [16-18]. Substantial literature data showed a link between adnexal mass and menopausal status [7]. Moreover, the incidence and mortality rate of ovarian cancer increases with patient age [14]. All these risk factors were also found to be significant for tumor triage in the patients studied. Achieved models showed that the most important for predicting malignancy out of all anamnestical data was menopausal status, from characteristics of women, tumors metastases, and from laboratory analyses RMI and ESR.

Serum CA-125 has been investigated for ovarian cancer screening with conflicting results [19]. CA-125 determination is useful for the detection of the persistence and recurrence and monitoring of the therapeutic effects in patients with epithelial ovarian carcinomas.

CA-125 is the most reliable serum marker in use for serial measurements to calculate the risk of cancer, which appears to have greater utility than evaluation of a single value [20]. However, elevated levels of CA-125 can also be detected in many non-malignant gynecological diseases and some physiological conditions. Numerous researchers have confirmed that CA-125 has limitations when used to distinguish between benign and malignant ovarian masses, but have concluded that by using likelihood reference tables, clinicians will be able to better interpret preoperative serum CA-125 results in patients with adnexal masses [5, 18, 20-22]. Regarding the results in this study, it can be concluded that, CA-125 is significantly correlated with HP findings, but some other tumor markers, like CEA and Ca 15.3, should also be taken into consideration.

Some studies suggest that transvaginal ultrasound (TVUS) can discriminate between benign and malignant ovarian tumors, better than all other radiological methods [4]. However, imaging techniques including TVUS evaluation alone have not fulfilled this goal [13]. This study showed that simple ultrasound findings - larger tumor dimensions, as well as the presence of metastases and ascites - can determine the malignancy of the tumor. In addition, multilocularity, bilaterality, and solid parts can implicate that the tumor is either malignant or borderline.

RMI is the most often used method for predicting the likelihood of malignancy of adnexal mass. It is derived from multivariate logistic regression analysis, incorporating menopausal status, ultrasonic score, and serum CA-125 levels. Each of these parameters has been shown to be significantly and independently related to the likelihood of malignancy [7]. Moreover, it comprises the majority of significant parameters and takes into consideration their relationships. Its effectiveness has been validated in a number of retrospective and prospective studies in which RMI had sensitivity of 85% and a specificity of 97% in differentiating malignant from benign diseases [7]. Although, some other more complex models available in literature also outperform the current reference test RMI - for discriminating between benign and malignant adnexal masses [23], RMI represents a low-cost, simple but highly-effective tool for triage in the management of women with adnexal masses [5, 7]. This study also showed that RMI can discriminate malignant and benignant as well as borderline tumors. Furthermore, Forward Wald method has proven to have the most importance for prediction of malignancy. The higher the RMI, the more probable is that the tumor is malignant. Moreover, the authors found that RMI can be considered simultaneously with the levels of ESR and CEA for more precise triage.

The established models in this study for AMI, CMI, and LMI were calculated, and proved to be linked with HP findings. Therefore, they can be introduced in preoperative evaluation of patients with adnexal tumors. Further studies can prove the validity of these new models by testing and retesting them in different populations, as well as to assess how to best implement these indices.

Conclusions

The achieved models showed that the most important for predicting malignancy out of all anamnestical data was menopausal status, from characteristics of women and tumors metastases, and from laboratory analyses RMI and ESR. Moreover, previous gynecological illnesses, patient age, and BMI have proved to be of great significance. RMI can be combined with ESR and CEA levels for better discrimination of malignant from other tumor types. Sensitivity of AMI was 73.33%, specificity 72.87%, PPV 39.49%, and NPV 91.88%. Sensitivity of CMI was 92.38%, specificity 67.36%, PPV 40.59%, and NPV 97.34%. Sensitivity of LMI was 56.45%, specificity 90.24%, PPV 68.63%, and NPV 84.57%. According to these results, the authors can advise introduction of these three indices (AMI, CMI, LMI) in clinical practice for more reliable differentiation of adnexal masses nature.

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Decreased prevalence of high-risk human papillomavirus infection is associated with obesity

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Summary

Purpose of investigation: Obesity is correlated with low education, low economic status, and lower rates of Pap smears, which are known as socio-demographic risk factors for cervical cancer. However, the association between obesity and high-risk human papillomavirus (HR-HPV) infection, the necessary cause of cervical cancer, and its related precursors, is not established. *Materials and Methods:* The authors examined the association between obesity and HR-HPV infection in 6,868 patients, who participated in annual health examinations at the Kangbuk Samsung Hospital in Seoul, Korea, from January through December 2007. *Results:* The prevalence of HR-HPV infection was 14.8%. Women infected with HR-HPV had a lower body mass index (BMI), when compared with non-infected women. After adjustment for alcohol intake, cigarette smoking, and marital status, HR-HPV infection was found to be negatively associated with BMI. When the analysis was stratified according to BMI, the risk of HR-HPV infection was significantly lower among those who were overweight (OR = 0.817, 95% CI = 0.680 – 0.982), or obese (OR = 0.688, 95% CI = 0.556 – 0.851), when compared with women with normal weight. *Conclusion:* HR-HPV infection was associated with obesity defined by BMI, with a lower prevalence of infection observed in obese women.

Key words: HPV; Obesity; Risk factor; Prevalence.

Introduction

Since the recognition of high-risk human papillomavirus (HR-HPV) as the causal agent of cervical cancer and its precursor lesions (cervical intraepithelial neoplasia) [1], a substantial amount of epidemiological data has revealed that a higher risk of HR-HPV infection, and progression to precursor lesions and cancer, were significantly associated with younger age, an increased number of sexual partners, and increased frequency of sexual intercourse, smoking, previous exposure to other sexually-transmitted diseases, high parity, contraceptive use, alcohol consumption, immunosuppressive conditions, lower mean income, and unmarried status [2-8]. In contrast, condom use reduced the risk of HPV infection [9].

To the authors' knowledge however, there are no recognizable studies to date addressing the association of HR-HPV infection and obesity. Obesity is a significant contributory factor to the development of gynecological cancer [10]. A strong association between obesity and endometrial cancer was demonstrated [10-12], which is assumed to be mediated by elevated estrogen levels following the aromatization of androstenedione in adipose tissue [10]. Although not all studies on this subject have led to identical results [12], some studies have reported that a significant increase in the risk for cervical cancer was observed in obese women [11]. Notably, obesity was strongly associated with adenocarcinoma, but not with squamous cell carcinoma of the uterine cervix [13].

Predictors of HR-HPV infection may differ from those of cervical cancer, because its development is influenced by many variables [8]. Obesity is correlated with low education and low economic status [14 ,15], which are known as socio-demographic risk factors for cervical cancer [8]. Moreover, obese women are less likely to adhere to physician recommendations for cervical cancer screening [16], which leads to lower rates of Pap smears in obese women [17]. Therefore, the relationship between obesity and HR-HPV infection warrants study.

Materials and Methods

The authors conducted a cross-sectional study of 6,868 patients, who participated in annual health examinations at the Kangbuk Samsung Hospital in Seoul, Korea, from January through December 2007. All subjects provided informed consent prior to participation in the study, which was approved by the ethical committee of the institution. Exclusion criteria included obesity secondary to hypothyroidism or Cushing's disease, severe debilitating diseases, cancer, or loss of more than 10% of normal weight during the previous six months. Women with a history of hysterectomy, and who had a Pap smear for a reason other than routine physical or pregnancy examination, were also excluded from the study.

Anthropometric measurements and blood chemistry

The authors measured the height and weight of every participant in the study. Body mass index (BMI; kg/m^2) was calculated using the measured weight and height. All blood samples were obtained after an overnight fast. The measurements of plasma glucose, cholesterol, triglycerides, and C-reactive protein (CRP) were evaluated using routine clinical chemistry methods. Insulin was measured by radioimmunoassay. Insulin

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Table 1. — *Characteristics of the study subjects according to HR-HPV infection status*.

	HR-HPV infection test negative (n = 5854)	positive (n = 1014)	*p* value[b]
Age (years)	42.54 ± 9.31	42.41 ± 9.37	0.688
BMI (kg/m^2)	22.19 ± 2.97	21.79 ± 2.72	< 0.001
Fasting plasma glucose (mg/dl)	92.57 ± 13.60	92.81 ± 17.02	0.609
Total cholesterol (mg/dl)	184.27 ± 32.66	183.13 ± 32.51	0.305
Triglycerides (mg/dl)	92.88 ± 53.84	93.09 ± 57.63	0.909
HDL-cholesterol (mg/dl)	55.44 ± 11.75	55.83 ± 12.20	0.331
LDL-cholesterol (mg/dl)	104.76 ± 28.59	103.35 ± 28.81	0.146
Fasting plasma insulin (μIU/ml)	9.62 ± 2.93	9.48 ± 2.49	0.095
HOMA-IR	2.22 ± 0.82	2.18 ± 0.73	0.169
CRP (mg/dl)*	0.04 ± 0.39	0.04 ± 0.38	0.893
Hypertension (%)	5.9	5.4	0.570
Diabetes mellitus (%)	1.3	0.9	0.258
Alcohol intake (%)			
Yes	34.7	40.1	0.001
No	65.3	59.9	
Cigarette smoking (%)			
Yes	5.1	7.2	0.006
No	94.9	92.8	
Exercise (%)			
Yes	51.1	49.8	0.442
No	48.9	50.2	
Marital status (%)			
Unmarried	3.3	6.1	< 0.001
Married	90.9	87.4	
Divorced, separated, widowed	5.8	6.5	

*Log transformation values.

resistance was estimated by homeostasis model assessment (HOMA); the HOMA insulin resistance index (HOMA-IR) was calculated using the following formula: fasting plasma glucose (mg/dl) × fasting plasma insulin ([IU/ml] / 405).

Detection of HPV DNA

The authors used the Hybrid Capture II system (Digene, Gaithersburg, MD, USA) for HR-HPV detection, according to the manufacturer's instructions. This technology is a nucleic acid hybridization assay where specimens containing the target DNA hybridize with a specific HPV RNA probe mixture containing probes for carcinogenic HR-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. This test was carried out using a luminometer and the results were expressed as relative light units (RLU). Any given sample was classified as positive when the RLU/positive control ratio, calculated as RLU of specimen/mean RLU of three positive controls, was 1 pg/ml or greater.

Statistical analysis

Data were reported as mean ± SD for continuous variables, and as numbers or a percentage for categorical variables. Clinical and biochemical characteristics of HR-HPV-infected and -uninfected women were compared using Student's t-test and chi-square test, when the variables were continuous or categorical, respectively. Because of skewed distributions, CRP levels were logarithmically transformed. Univariate logistic analyses were used to calculate the crude odds ratios (ORs) for potential risk factors associated with HR-HPV infection. Significant variables in univariate analyses were entered into multivariate logistic regression models to calculate adjusted ORs (95% CI).

Table 2. — *Univariate and multivariate odds ratios (ORs) for HR-HPV infection*.

	Crude OR (95% CI)	*p* value	Adjusted OR** (95% CI)	*p* value
BMI (kg/m^2)	0.953 (0.931 – 0.976)	< 0.000	0.954 (0.931 – 0.977)	< 0.000
Alcohol intake*	1.261 (1.100 – 1.446)	0.001	1.198 (1.042 – 1.378)	0.011
Cigarette smoking*	1.446 (1.110 – 1.885)	0.006	1.256 (0.956 – 1.650)	0.102
Marital status				
Married	1		1	
Unmarried	1.919 (1.430 – 2.577)	0.000	1.686 (1.248 – 2.279)	0.001
Divorced, separated, widowed	1.169 (0.890 – 1.536)	0.262	1.266 (0.959 – 1.672)	0.096

*References of risk factor: Never. **ORs were adjusted for all other covariates in the model.

For *post hoc* analysis according to BMI, the subjects were categorized into the following groups, based on Western Pacific Region World Health Organization (WHO) criteria on obesity: "underweight" if the BMI was less than 18.4, "normal" if the BMI was 18.5 to 22.9, "overweight" if the BMI was 23.0 to 24.9, and "obese" if the BMI was over 25.0 [18]. The statistical significance of trends for ORs was assessed by considering the categorical variable as a continuous variable in the logistic model.

All reported *p* values were two-tailed and considered significant when $p < 0.05$. Statistical analyses were performed using SPSS software, version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

The prevalence of HR-HPV infection in the population under study was 14.8%. The characteristics of the study subjects according to HR-HPV infection status are presented in Table 1. Women infected with HR-HPV had a lower BMI and higher prevalence of alcohol intake, smoking, and unmarried status; however, no difference was observed between the two groups regarding the lipid profile and CRP levels.

Age-specific prevalence of HR-HPV infection is shown in Figure 1; the prevalence of HR-HPV infection was not significantly associated with age ($p = 0.903$).

The multivariate logistic regression analysis identified BMI, alcohol intake status, and marital status as independent risk factors for HR-HPV infection (Table 2). In order to investigate the risk for HR-HPV infection associated with obesity, the latter was stratified according to BMI. In comparison to normal-weight women, overweight women had a lower risk of HR-HPV infection (OR = 0.817, 95% CI = 0.680 – 0.982) and obese women also had a lower risk of HR-HPV infection (OR = 0.688, 95% CI = 0.556 – 0.851); however, there was no significant difference in risk for HR-HPV infection between normal weight and underweight women (Table 3).

Discussion

To the best of the authors' knowledge, the study presented here is the first to report an association between

Table 3. — *Univariate and multivariate odds ratios (ORs) for HR-HPV infection according to BMI.*

BMI (kg/m²)	HR-HPV test		Crude OR (95% CI)	*p* value	Adjusted OR* (95% CI)	*p* value
	negative	positive				
18.5 – 22.9	3402 (58.1)	648 (63.9)	1		1	
< 18.5	449 (7.7)	78 (7.7)	0.912 (0.707 – 1.177)	0.479	0.910 (0.705 – 1.175)	0.471
23 – 24.9	1099 (18.8)	170 (16.8)	0.812 (0.677 – 0.974)	0.025	0.817 (0.680 – 0.982)	0.031
≥ 25	904 (15.4)	118 (11.6)	0.685 (0.556 – 0.845)	< 0.001	0.688 (0.556 – 0.851)	0.001
			p for trend < 0.001		*p* for trend < 0.001	

*ORs were adjusted for alcohol intake, cigarette smoking, and marital status.

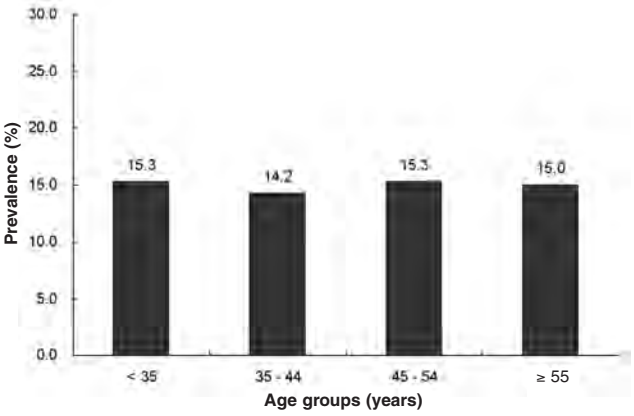

Figure 1. — The prevalence of HR-HPV infection by age of the subject.

obesity and HR-HPV infection in Korean women. It was found that BMI was significantly correlated with HR-HPV infection and that the risk for HR-HPV infection was decreased in accordance with obesity. Overweight status was associated with a 0.817-fold decreased risk for HR-HPV infection and obesity was associated with a 0.688-fold decreased risk for HR-HPV infection, even after adjusting for confounding variables.

The authors had hypothesized, *a priori*, that obese women would have an increased risk for HR-HPV infection. Interestingly, however, the findings in this study suggest the opposite effect and the association detected here cannot be easily explained.

There are several explanations that can be considered. First, obesity may cause changes in sexual behaviors. In men, both overweight and obesity statuses have been identified as risk factors for sexual dysfunction [19] and in women, obesity was also associated with impairment of sexual function, including arousal, lubrication, satisfaction, orgasm, and sexual interest [20-22]. Obese women reported greater impairments of sexual enjoyment, desire, and avoidance of sexual encounters than obese men [23]. Another study reported that moreover, slimmer people of both genders are generally more sexually-attractive, and in healthy women aged 19–40 years, hip size was negatively associated with the frequency of penile-vaginal intercourse [24]. Thus, for these reasons, obese women seem to have lower sexual activity. As sexual activity is the most important primary risk factor for HPV infection, it can be extrapolated that the risk for HR-HPV infection is lower in obese women because of decreased sexual activity.

Another possible explanation of the current findings is a change in immunity. Most HR-HPV infections are subclinical and self-limited [25]; however, a small but medically important fraction of the lesions will progress to cervical cancer [26]. In addition, the prevalence of HR-HPV infection increased, and lesions progressed more rapidly to cancer, among immunosuppressed populations such as those infected with the human immunodeficiency virus (HIV) or renal transplant recipients [2, 3, 25]. These observations suggest an important, albeit currently unknown role of immune responses in the control of HR-HPV infection and progression to cancer.

Cytokines play an important role in the defense against HR-HPV infection, modulating viral replication, and polarizing the immune response to the Th1 or Th2 patterns [27]. Among many cytokines, tumor necrosis factor-α (TNF-α) induces cell-mediated immunity and plays a striking role in viral clearance and inflammatory reactions [28]. TNF-α has specific antiviral effects on HR-HPV through repression of its gene transcription [29]; however, the repression of HR-HPV by TNF-α is lost during malignant conversion [30]. TNF-α is increased in the serum of obese subjects [31]. Moreover, two previous studies found that TNF-α levels were correlated in sera and cervical secretions in fertile and infertile women [32, 33]. In conclusion, the change in immunity in obese women, including changes in serum TNF-α levels, may cause alterations in local cervical immunity, which in turn may lead to a decrease in HR-HPV infection. Other studies have reported that the immune responses may differ between systemic and local infections, at least in terms of cytokine levels [34]; in particular, intralesional TNF-α levels were found to be not associated with HPV-16 infection [35, 36]. In contrast to normal and HPV-16-immortalized keratinocytes, which were sensitive to TNF-α, HPV-18-immortalized keratinocytes were resistant to the inhibitory effects of this cytokine [37]. Therefore, the effect of TNF-α can vary according to HPV type. In addition, other adipocytokines (e.g., leptin) related to obesity may also play a role. Further studies are necessary to clarify this issue.

In the present study, the prevalence of HR-HPV infection was 14.8%, which is similar to that previously reported for Korean [4, 38] and American women [8, 39].

The prevalence of HPV infection was reported to be highest among females below 25 years of age [8, 39] however, in this study the authors did not observe a sig-

nificant difference in HPV infection between age groups. This discrepancy may be explained partly by the low recruitment of women under 25 years of age (n = 26) in this study [40].

Women who consumed alcohol had an increased risk of HR-HPV infection, when compared with never-drinkers. Unmarried women also had an increased risk of HR-HPV infection. These results are in agreement with findings of previous studies [4, 8].

There are several limitations in this study. First, a cross-sectional design was used, which means that causality could not be determined. Individuals infected with multiple pathogens, such as herpes simplex virus, cytomegalovirus, and Helicobacter pylori, have high CRP levels (a marker of inflammation), as well as an increased risk for coronary artery disease [41], and the metabolic syndrome [42]. The phenomenon may be due to the multiple pathogens that induce production of proinflammatory cytokines, which in turn leads to chronic subclinical inflammation and the metabolic syndrome [42]. Polterauer et al. reported that inflammation-induced cytokines may play a role in cervical carcinogenesis, tumor progression, and cancer prognosis [43]. Serum CRP levels can also be used as an additional prognostic parameter in patients with cervical cancer. However, HPV infection is highly-localized to the squamous epithelium, without significant systemic manifestations [25]. The median duration of HPV infection is eight months and about 90% resolve within two years [6]. Although genital infection with HPV is followed by serologic response, a substantial proportion of HPV-infected women fail to seroconvert [8]. In the present study, there was no significant difference in CRP levels between the two groups, and no correlation with the metabolic syndrome was observed (data not shown). Therefore, it is very unlikely that HR-HPV infection acted as the cause of obesity in these women.

Second, the authors observed a relationship between obesity and HR-HPV infection, but obesity is not known as a risk factor for cervical cancer. The results gathered in this study are not sufficient to confirm the influence of the relationship between obesity and HR-HPV on findings of cytology and progression to cancer. These differences may reflect the time interval between infection and development of cancer, as well as the many other variables that influence the development of cancer [8]. Therefore, additional studies are necessary to resolve this issue.

In conclusion, HR-HPV infection was influenced by obesity, with a lower prevalence of infection observed in obese women.

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Characteristics and prognosis of ovarian metastatic tumors: review of a single-institution experience

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Summary

Background: To evaluate the clinico-pathological characteristics and role of surgery in patients with ovarian metastasis. *Materials and Methods:* Clinical data from 51 patients with pathologically confirmed ovarian metastasis were reviewed. *Results:* Ovarian metastasis accounted for 14% of all malignant ovarian neoplasms (51/364). Of the 51 metastatic ovarian tumor cases, 24 originated from gynecologic malignancies, while 27 originated from non-gynecologic malignancies. Optimal cytoreduction was performed in 88% and 37% of patients with metastases of gynecologic and non-gynecologic origin, respectively. Patients with ovarian metastasis had a two-year survival rate in 82% of the gynecologic group and 70% of the non-gynecologic group ($p = 0.35$). The five-year survival rate of patients with non-gynecologic tumor origin (29%) was significantly worse ($p = 0.04$) than the survival rates of those with tumors of gynecologic origin (61%). In the non-gynecologic group, the five-year survival rates were significantly different between patients who were performed optimal cytoreductive surgery vs those without this procedure (42% and 20%, respectively; $p = 0.04$). *Conclusion:* Although complete surgical resection is not achievable in approximately two-thirds of patients with metastases of non-gynecological origin, optimal tumor cytoreduction appears to improve survival, which is statistically significant in all patients with ovarian metastatic tumors.

Key words: Ovarian metastasis; Cytoreductive surgery; Krukenberg tumor.

Introduction

The ovaries are frequent sites of metastasis for malignancies originating from gynecologic and non-gynecologic areas. Metastatic ovarian tumors account for 5%-30% of all malignant ovarian tumors [1]. This frequency seems to be associated with the prevalence and spreading patterns of primary malignancy. The majority of non-gynecologic cancer metastasis to the ovaries originates from the gastrointestinal (GI) tract and breasts [2]. Another frequent primary site is the gynecologic tract. In many cases, it can be difficult to distinguish between primary and metastatic ovarian tumors. Some authors reported that 45% of metastatic ovarian tumors originating from colon cancer were misdiagnosed as primary ovarian cancer due to the mucin-producing pattern of these tumors [2, 3]. Additionally, ovarian metastasis is commonly detected before primary tumor [4]. Distinguishing between primary and metastatic ovarian tumors is important because misinterpretation may lead to inappropriate management and suboptimal treatment outcomes.

According to previous studies, tumors of non-gynecologic origin with ovarian metastasis have worse prognosis than tumors of gynecologic origin with ovarian metastasis and primary ovarian cancer [5, 6]. Although cytoreductive surgery and adjuvant chemotherapy have a proven role in primary epithelial ovarian cancer, there is limited information regarding the benefits of cytoreductive surgery in patients with metastatic ovarian tumors of gynecologic and non-gynecologic origin. The purpose of this study was to assess the clinical characteristics, to evaluate the role of surgery, and to compare the survival rates in 51 cases of metastatic ovarian tumors of gynecologic and non-gynecologic origin.

Materials and Methods

The Institutional review board approved this study. A total of 51 patients with ovarian metastasis from gynecologic and non-gynecologic primary sites were identified at the Bakirkoy Woman and Children Training and Research Hospital between 2002 and 2010. During this time, 313 cases of primary malignant ovarian tumors were resected and histopathologically diagnosed. The available clinical records of patients with ovarian metastasis were reviewed for age, menopausal status, clinical manifestation of metastasis, serum CA-125 levels, intraoperative findings, primary site of origin, adjuvant treatment, and the effect of surgery on survival. The distinction between primary and metastatic tumors was based on the diagnostic approach of Young and Scully [7]. The diagnosis of ovarian metastasis was confirmed by histological and immunohistochemical stains (cytokeratin 7 and 20) in selected cases. The categorized surgical modalities are as follows: (1) total abdominal hysterectomy, bilateral salpingo-oophorectomy (TAH+BSO), omentectomy, and bilateral pelvic and para-aortic lymphadenectomy (PPALND) with complete cytoreduction; (2) TAH+BSO and omentectomy with incomplete removal of the primary tumor; and (3) minimal surgical effort including salpingo-oophorectomy, wedge resection, or biopsy of ovarian masses. Cytoreductive surgery was defined as optimal when the largest residual tumor mass was less than one cm.

Statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Pearson's chi-square or Fisher's exact test was used to analyze the categorical variables. Survival analyses were estimated by the Kaplan-Meier method, and dif-

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ferences between groups were calculated using the log-rank test. All intervals were determined from the date of surgery. The results were considered statistically significant if the p value was < 0.05.

Results

During the nine-year study period, 51 cases of ovarian metastases and 313 cases of primary ovarian malignancies, including synchronous primary ovarian tumors, were identified at the Institutions. According to these data, ovarian metastases comprise 14% (51/364) of all malignant ovarian neoplasms. The mean age of the study group was 50.8 ± 12.2 years (range 29 - 72). Of the 51 metastatic ovarian tumor cases, 24 (47%) originated from gynecologic malignancies while 27 (53%) originated from non-gynecologic malignancies. Metastatic non-gynecologic tumors occurred in a slightly younger age group than metastatic gynecologic tumors (49.3 vs 52.5). Postmenopausal patients accounted for 41% and 46% of the non-gynecologic and gynecologic groups, respectively. The most common presenting symptoms of tumors of non-gynecologic origin were increased abdominal girth (63%) and abdominal/pelvic pain or discomfort (26%); however, abnormal uterine bleeding (58%) was the most commonly seen symptom in malignancies of gynecologic origin. Serum CA-125 was used as a tumor marker for most patients (84%) in this study. The median serum CA-125 level was 245 U/ml (range 14 - 1,352) and 348 U/ml (range 13 - 5,734) for metastatic ovarian tumors of gynecologic origin and non-gynecologic origin, respectively ($p = 0.19$).

In the gynecologic group, uterine corpus was the most common primary site (29.4%), followed by uterine cervix (9.8%), and Fallopian tube (7.8%). Fourteen cases of uterine corpus tumors were adenocarcinomas (six endometrioid, three serous, three clear cell, and two otherwise unspecified), while one case was endometrial stromal sarcoma. The histological types of uterine cervical cancers included two adenocarcinomas and three squamous cell carcinomas. Additionally, four cases of Fallopian tube malignancies with ovarian metastasis consisted of one Wolffian tumor and three cases of tubal adenocarcinoma. In the non-gynecologic group, anatomic locations of primary tumors were as follows: colon (25%), stomach (9.8%), breast (5.9%), and appendix (3.9%). There were also tumors of unknown primary origin (9.8%), but most had histopathological features resembling an upper GI tract or colon origin (Table 1). Mucin-producing adenocarcinomas were the most commonly identified histological type of non-gynecologic metastatic tumors (70%). However, a signet-ring cell component was found in six of all ovarian metastases (12%) and accounted for 19% and 4% of non-gynecologic and gynecologic metastatic ovarian tumors, respectively.

Bilateral ovarian involvement was present in 33% of the gynecologic group and 74% of the non-gynecologic group ($p = 0.01$). On the other hand, a minimum of unilateral prominently-enlarged ovarian masses were observed in 71% of metastases of gynecologic origin and 96% of metastases of non-gynecologic origin, proving that occult metastasis was present in 29% of the gynecologic group and 4% of the non-gynecologic group. The sizes of the ovaries in the metastatic disease were recorded in 43 out of 51 patients. The mean sizes of the largest masses for the gynecologic and non-gynecologic groups were 5.9 cm (range 3 - 10) and 7.1 cm (range 3 - 12), respectively ($p = 0.13$). Demographics, clinical and pathological features, as well as the differences of the constituent ratio are compared between these two subgroups in Table 2.

Extensive surgical resection was performed in 88% of gynecologic origin metastatic tumors (21/24). In the non-gynecologic group, TAH + BSO, omentectomy, and PPALND with complete cytoreduction were performed in ten patients (37%); 14 patients (52%) had TAH + BSO + omentectomy with incomplete removal of the primary tumor; and three patients (11%) had minimal surgical effort, including salpingo-oophorectomy, wedge resec-

Table 1. — *Primary sites of ovarian metastatic tumors*.

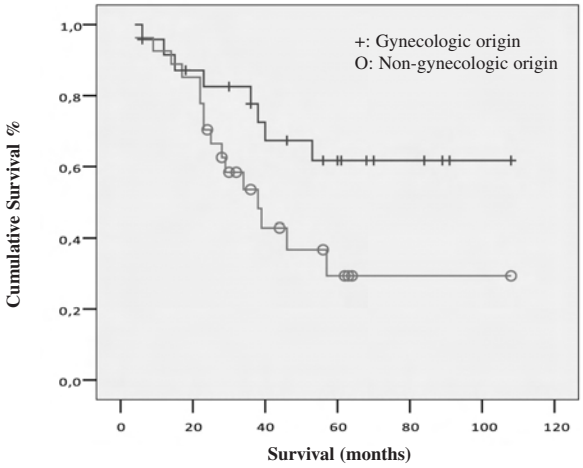
Site	No. of patient (%)
Non-gynecologic origin	27 (52.9)
Colorectal	12 (25)
Stomach	5 (9.8)
Breast	3 (5.9)
Appendix	2 (3.9)
Tumor of unknown origin	5 (9.8)
Gynecologic origin	24 (47.1)
Endometrium	14 (27.4)
Cervix	5 (9.8)
Fallopian tube	4 (7.8)
Sarcoma	1 (2)

Table 2. — *Clinico-pathologic characteristics of patients with metastatic ovarian tumors*.

	Non-gynecologic origin (n = 27)	Gynecologic origin (n = 24)	p value
Age (mean ± SD)	49.3 ± 12.6	52.5 ± 11.7	0.27
Postmenopausal	41%	46%	0.73
Symptom			
Abdominal distension	63%	29%	
Abdominal/pelvic pain	26%	8%	
Abnormal uterine bleeding	7%	58%	
Asymptomatic	7%	8%	
Ascites			
Positive	89%	46%	0.002
CA-125 (median)	348 U/ml	245 U/ml	0.19
Bilaterality	74%	33%	0.01
Occult metastases	4%	29%	0.02
Mean sizes of the largest masses	5.9 cm	7.1 cm	0.13
Surgery types			
Extensive surgery*	37%	88%	
TAH+BSO	52%	12%	
Minimal surgical effort†	11%		

* Total abdominal hysterectomy, bilateral salpingo-oopherectomy, omentectomy, pelvic and para-aortic lymphadenectomy, and complete debulking of the tumor.
† Salpingo-oopherectomy, wedge resection, or biopsy of ovarian masses.

Fig. 1



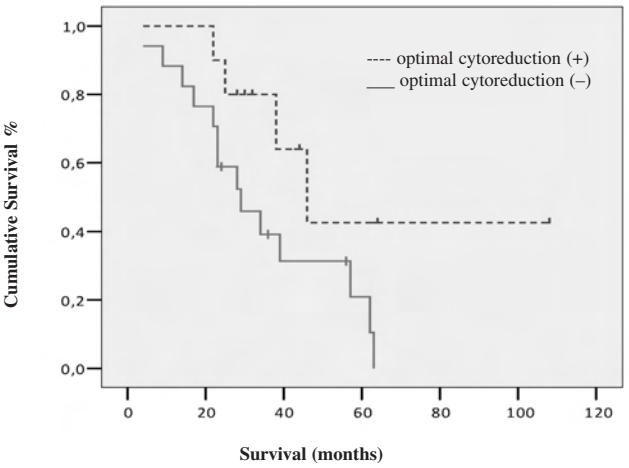


Fig. 2

Figure 1. — Survival curves of patients with metastatic ovarian tumors according to primary origin.
Figure 2. — Survival curves of patients with non-gynecologic origin metastatic ovarian tumors according to cytoreductive surgery.

tion or biopsy of ovarian masses. Colon resection was performed in five out of 14 patients with appendix-colorectal cancer during initial cytoreductive surgery (36%). Of 27 patients, ten (37%) were optimally cytoreduced, and 17 (63%) had suboptimal residual disease. All patients received adjuvant therapy; 69% had chemotherapy alone, 10% had radiotherapy alone, and 21% had chemotherapy and radiotherapy combined after surgery.

Follow-up began with the surgery of metastatic ovarian tumors, and the mean follow-up for these tumors was 43 months. The overall two- and five-year survival rates of 51 patients was 77% and 53%, respectively. Patients with ovarian metastasis had a two-year survival rate of 82% in the gynecologic origin group and 70% in the non-gynecologic group; however, the difference in two-year survival rates was not statistically significant ($p = 0.35$). The five-year survival rate of patients with a tumor of non-gynecologic origin (29%) was significantly worse ($p = 0.04$) than patients with a tumor of gynecologic origin (61%). The median survival time for patients who underwent optimal cytoreductive surgery was significantly superior to those who had suboptimal cytoreductive surgery (46 vs 29 months) in the non-gynecologic group. The five-year survival rates were significantly different between patients with and without cytoreductive surgery in the non-gynecologic group (42% and 20%, respectively; $p = 0.04$). Survival rates of patients are shown in Figures 1 and 2.

Discussion

The most common primary cancers that metastasize to the ovaries in the Western series are: colon, followed by stomach, breast, and gynecologic cancers [8], whereas stomach cancer was common in Eastern Asia and especially in Japan [9]. In this current study, metastatic ovarian tumors accounted for 14% of all ovarian malignancies and the GI tract (colorectal-appendix 28.9%, stomach 9.8%) was the most common primary site. These findings are consistent with previous studies. Webb *et al*. reported the GI tract as the primary site in 47%, breast in 31%, and gynecologic organs in 18% of cases [8]. Ulbright *et al*. demonstrated a 7% incidence of ovarian metastasis in patients who were thought to have a primary ovarian malignancy and also reported gastric cancers contributed 8% of metastatic ovarian lesions, similar to 9.8% seen in this series [2].

The mean age was 50.8 years in this study. Metastatic tumors of non-gynecologic origin occurred in a slightly younger age group than those with metastatic tumors of gynecologic origin (49.3 vs 52.5, respectively, $p = 0.27$). Although both gynecologic and GI malignancies are seen in later decades of life, it is quite concerning that patients with ovarian metastasis are rather a younger population. In the authors' opinion, there might be a relation between higher blood flow to the ovaries in earlier decades of life and increased risk of metastasis.

Occasionally, metastatic ovarian tumors morphologically and clinically resemble a primary ovarian neoplasm [10]. Furthermore, the radiological features of metastatic ovarian cancer show considerable variability. In this series, although the majority of patients underwent additional radiological assessment (65%) and gastroscopic/colonoscopic examination (25%), primary tumors of non-gynecologic group were known prior to resection of ovarian metastasis in only 11% of cases. Ayhan *et al*. [5] reported that the clinical diagnosis of primary tumor preceded the metastatic ovarian lesion in only 30% of patients and were mostly in patients with breast cancer (86%).

The incidence of bilaterality in metastatic ovarian cancers ranged from 54% to 70% [2, 5, 11]. In this series, the bilaterality ratio was 33% and 74% for gynecologic and non-gynecologic origin tumors, respectively ($p = 0.01$). Although bilaterality is far more frequent in the non-gynecologic group, occult metastasis was only 4%. The

reason for rarity of occult metastases in patients with tumors of non-gynecologic origin is that many of these were in advanced stages. On the other hand, it was significantly higher in metastatic tumors of endometrial (29%, 4/14) and cervical (40%, 2/5) origin.

For metastatic tumors of gynecologic origin, uterine corpus was the most common primary site. The frequency of metastatic endometrial adenocarcinomas was double that of cervical and tubal metastatic cancers combined. During the nine-year period of data collection, there were 304 cases of endometrial adenocarcinomas and 144 cases of uterine cervical cancers (112 cases of squamous cell carcinoma, and 32 cases of adenocarcinoma). The rates of ovarian metastasis in endometrial adenocarcinoma and uterine cervical cancer were 4.6% and 3.4%, respectively.

Although metastatic ovarian tumors are frequently called Krukenberg tumors, in this study, the authors have used the original definition of Woodruff and Novac: "a tumor arising in the ovarian stroma having characteristic mucin-filled, signet-ring cells" [12]. Krukenberg tumors accounted for 12% (6/51) of metastatic ovarian tumors; the primary tumor was most frequently located in the stomach (5/6). Song *et al*. [13] and Cheong *et al*. [14] reported that the incidence of the stomach as primary site of a Krukenberg tumor were 70% and 94%, respectively, which is consistent with the results of this current study (83%).

Ovarian metastases from other primary sites are manifestations of advanced disease; thus, the prognosis is generally poor. Petru *el al*. reported that the overall actuarial five-year survival rate was 10% in patients with non-gynecologic metastatic tumor [4]. In this present series, the overall five-year survival rate of patients with metastatic ovarian tumors originating from gynecologic and non-gynecologic organs were significantly different (61% and 29%, respectively; $p = 0.04$). This poor survival rate is consistent with the Yada-Hashimoto's study [1] which reports that the five-year survival rate of patients with metastatic ovarian tumors originating from gynecologic and non-gynecologic organs were 47% and 19%, respectively.

The extent of disease, biologic aggressiveness of the tumor, and the presence of a complete surgical resection have significant roles in the survival of patients with primary ovarian tumors. Nevertheless there is limited information regarding the outcome of patients with ovarian metastatic tumors that undergo cytoreduction. Some reports compared the survival rates of complete surgical resection vs palliative approach, especially in patients with colorectal cancer with ovarian metastasis. In these reports, better survival rates were demonstrated in cases that underwent complete debulking [2, 15]. In contrast, Miller *et al*. suggested that tumor reduction should be avoided and a palliative approach should be taken [10]. In this present series, cytoreductive surgery seemed to be beneficial in all patients with ovarian metastatic tumors. The median survival time was significantly superior in patients who underwent optimal cytoreductive surgery compared to those who underwent suboptimal cytoreductive surgery (46 vs 29 months) in the non-gynecologic group. As well, the five-year survival rates were significantly different between patients with and without optimal cytoreductive surgery in the non-gynecologic group (42% and 20%, respectively; $p = 0.04$).

In conclusion, although complete surgical resection is not achievable in approximately two-thirds of patients with metastases of non-gynecological origin, optimal tumor cytoreduction appears to improve the survival rate, which is statistically significant in all patients with ovarian metastatic tumors. Nevertheless, patients with gynecologic tumor origin have significantly higher survival rates than patients with non-gynecologic tumor origin.

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Effects of loop electrosurgical excision procedure or cold knife conization on pregnancy outcomes

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Summary

Purpose: To explore the effects of cervical loop electrosurgical excision procedure (LEEP) or cold knife conization (CKC) on pregnancy outcomes. *Materials and Methods:* Patients with cervical intraepithelial neoplasia (CIN) who wanted to become pregnant and received LEEP or CKC were considered as the treatment groups. Women who wanted to become pregnant and only underwent colposcopic biopsy without any treatments were considered as the control group. The pregnancy outcomes were observed and compared in the three groups. *Results:* Premature delivery rate was higher ($p = 0.048$) in the CKC group (14 / 36, 38.88%) than in control group (14 / 68, 20.5%) with a odds ratio (OR) of 2.455 (1.007 - 5.985); and premature delivery was related to cone depth, OR was significantly increased when the cone depth was more than 15 mm. There was no significant difference in premature delivery between LEEP (10 / 48, 20.83%) and the control groups. The average gestational weeks were shorter ($p = 0.049$) in the CKC group (36.9 ± 2.4) than in the control group (37.8 ± 2.6), but similar in LEEP (38.1 ± 2.4) and control groups. There were no significant differences in cesarean sections between the three groups. The ratio of neonatal birth weight less than 2,500 g was significantly higher ($p = 0.005$) in the CKC group (15/36) than in the control group (10/68), but similar in the LEEP and control groups. *Conclusion:* Compared with CKC, LEEP is relatively safe. LEEP should be a priority in the treatment of patients with CIN who want to become pregnant.

Key words: Conization of cervix; Cervical loop electrosurgical excision procedure; Cervical cold knife conization; Cervical intraepithelial neoplasia; Pregnancy outcomes.

Introduction

Cervical intraepithelial neoplasia (CIN) is a group of pre-cancerous lesions closely related to invasive cervical cancer. In recent years, with the development of cervical screening technology, many patients with CIN have been identified in time and most of these patients are at a childbearing age and want to become pregnant. At present, loop electrosurgical excision procedure (LEEP) and cold knife (CKC) conization are commonly used in the treatment of CIN. A great deal of attention has been paid to the effects of surgical procedures on pregnancy outcomes. There have been different reports regarding the effects of cervical conization on pregnancy outcomes. Most studies from China have described that LEEP and CKC have no adverse effects on pregnancy outcomes, but these studies are retrospective analyses with small-scale cases and without controls [1, 2]. Foreign large-scale, multi-centric case-control analyses and meta-analyses have indicated that LEEP and CKC have certain adverse effects on pregnancy outcomes [3-5], however, the latter have not been compared between LEEP and CKC. In this study, a contract analysis of pregnancy outcomes was performed in 84 patients who became pregnant after LEEP (48 patients) or CKC (36 patients) and 68 women who became pregnant after only colposcopic biopsy between January 2005 and January 2009 in this Hospital.

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Materials and Methods

All study methods were approved by the Institutional Review Board and Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All the subjects enrolled into the study gave written formal consent to participate.

The patients who were diagnosed with CIN by colposcopic biopsy and postoperative pathology, who underwent LEEP or CKC, and who wanted to become pregnant between January 2005 and January 2009 were enrolled in this Hospital study. All patients had no histories of infertility and recurrent miscarriage, no evidence of premature delivery, and no smoking habits. They were followed for two years. There were 48 patients who became pregnant after LEEP with a mean age of 29.8 ± 5.1 years (range 20 - 39). Of the 48 patients, 12 had CIN I, 23 had CIN II, and 13 had CIN III. There were 36 patients who became pregnant after CKC with a mean age of 31.8 ± 3.8 years (range 22 - 38). Of the 36 patients, 11 had CIN II and 25 had CIN III. At the corresponding time period, 68 women aged 31.0 ± 4.0 years (range 21 - 39) who became pregnant after exclusion of CIN II or above CIN II by colposcopic biopsy and did not receive any surgical procedures, served as the control group. These women also had no histories of infertility and recurrent miscarriage, no evidence of premature delivery, and no smoking habits. They were followed for two years.

LEEP or CKC was performed by the same three surgical experts three to seven days after menstruation. The extent of cervical lesions was observed with compound iodine solution. Circumcision or conization was performed from 2 - 5 mm outside the iodine-unstained extent with an excision depth of 6 - 22 mm. The excisional tissue was marked for localization and the cone height was recorded, followed by pathological examination. Electric coagulation or transfixion was used to stop bleeding on the postoperative wound surface. The vagina was filled with gauze which was removed 24 hours later. Antibiotics were

routinely used to prevent infection. If the endovaginal examination was normal at three to six months postoperatively, the patients were permitted to consider pregnancy. The time, status, and outcomes of pregnancy were recorded. In the control group, the time, status, and outcomes of pregnancy after colposcopic biopsy were also recorded. The subjects who had postoperative infertility, early pregnancy abortion, ectopic pregnancy, multiple-time conization or positive incisal edge were excluded from this study.

Statistical treatment was performed with the SPSS 17.0 software. The chi-squared (χ^2) test was used in numeration data. Logistic regression analysis was used in premature delivery-related factor analysis.

Results

There were no statistical differences between the treatment groups and control group with regards to age, gravidity, and premature delivery-related complications including placenta previa, pre-eclampsia, pregnancy associated with diabetes, and bigeminal pregnancy (Table 1).

In the two treatment groups, there was no abortion during the second trimester. In the LEEP group, premature delivery occurred in ten patients with a premature delivery rate of 20.83%. Of the ten patients, four had preterm premature rupture of the membrane (PPROM), and two had premature delivery before the 34th gestational week. Term-delivery occurred in 38 patients in the LEEP group. In the 38 patients, the gestational week was ≥ 37 weeks and < 38 weeks in ten patients, ≥ 38 weeks and < 39 weeks in eight patients, ≥ 39 weeks and < 40 weeks in ten patients, and ≥ 40 weeks and < 42 weeks in ten patients. No post-term pregnancy occurred and the average gestational week was (38.1 ± 2.4) weeks in LEEP group. In the CKC group, premature delivery occurred in 14 patients with a premature delivery rate of 38.88%. Of the 14 patients, five had PPROM, and three had premature delivery before the 34th gestational week. Term-delivery occurred in the 22 patients in the CKC group. In 22 patients the gestational week was ≥ 37 weeks and < 38 weeks in six patients, ≥ 38 weeks and < 39 weeks in eight patients, ≥ 39 weeks and < 40 weeks in six patients, and ≥ 40 weeks and < 42 weeks in two patients. No post-term pregnancy occurred and the average gestational week was 36.9 ± 2.4 weeks in the CKC group. In the control group, premature delivery occurred in 14 women with a premature delivery rate of 20.59%. Of the 14 women, six had PPROM and one of the six PPROM occurred during the second trimester, and three had premature delivery before the 34th gestational week. Term-delivery occurred in 54 women in the control group. In 54 women, the gestational week was ≥ 37 weeks and < 38 weeks in 13 patients, ≥ 38 weeks and < 39 weeks in 14 patients, ≥ 39 weeks and < 40 weeks in 16 patients, and ≥ 40 weeks and < 42 weeks in 11 patients. No post-term pregnancy occurred and the average gestational week was (37.8 ± 2.6) weeks in the control group. The results are shown in Figure 1 and in Table 2. The premature delivery rate was higher in the CKC group than in the control group ($p = 0.048$) with a odds ratio (OR) of 2.455 (1.007 - 5.985). The average gestational week was lower in the CKC group than in the control group ($p = 0.049$).

The constituent ratios of the gestational week in LEEP, CKC, and control groups are shown in Figure 1. It can be seen from Figure 1 that the constituent ratios of gestational week are similar in the LEEP and control groups; but less than 37 gestational weeks was significantly greater ($p < 0.05$) in the CKC group (14/36) than in the control group (14/68), and 40-42 gestational weeks was less in CKC group (2/36) than in the control group (11/68) without statistical significance ($p = 0.211$).

Premature delivery-related factors including cone depth, cone area, and lesion severity were analyzed. Premature delivery was strongly related to cone depth (Table 3).

Further analysis indicated that less than 10 mm cone depth scarcely increased the risk of premature delivery; a 15 mm cone depth had an OR of 1.259 and more than 15 mm cone depth markedly increased OR (Figure 2).

Table 1. — *Comparison of the general data between treatment groups and control group*.

	LEEP group n = 48	CKC group n = 36	Control group n = 68	Test statistics and p values
Age (years)	29.8 ± 5.1	31.8 ± 3.8	31.0 ± 4.0	$\chi^2 = 0.651$, $p < 0.862$
Primiparity	36	26	48	$\chi^2 = 0.276$, $p < 0.871$
Complications	9	8	14	$\chi^2 = 0.156$, $p < 0.925$
Placenta previa	2	2	4	
Pre-eclampsia	3	2	4	
Diabetes	2	2	3	
Bigeminal pregnancy	2	2	3	

Table 3. — *Logistic analysis of premature delivery-related factors*.

	B	S.E.	Wald	df	Sig.	Exp (B)
CIN severity			5.098	2	0.078	
CIN (I)	- 2.861	1.427	4.019	1	0.045	0.057
CIN (II)	- 1.048	0.826	2.904	1	0.088	0.245
Excision depth	0.682	0.163	17.604	1	0.000*	1.979
Excision area	0.929	0.780	1.420	1	0.233	0.549

B = coefficient; S.E. = standard error; Wald = Chi-square value; df = degree of freedom; Sig. = p value; Exp (B) = OR value. * indicates statistical significance.

Table 2. — *Gestational weeks and hazard ratios in each group*.

Gestational weeks	LEEP group N=48	CKC group n = 36	Control group n = 68	OR values 95% CI LEEP group	CKC group
< 37 weeks	10 (20.83%)	14 (38.89%)*	14 (20.58%)	1.015 (0.408 ~ 2.525)	2.455 (1.007 ~ 5.985)
< 34 weeks	2 (4.16%)	3 (8.57%)	4 (5.88%)	0.696 (0.122 ~ 3.960)	1.455 (0.307 ~ 6.886)
PPROM	4 (8.33%)	5 (13.89%)	6 (8.82%)	0.939 (0.250 ~ 3.527)	1.667 (0.472 ~ 5.892)

* indicates $p < 0.05$ compared with the control group. PPROM: preterm premature rupture of membrane.

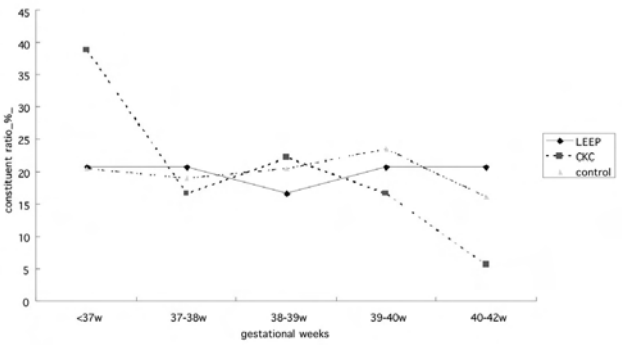

Figure 1. — The constituent ratios of gestational weeks in LEEP, CKC, and control groups.

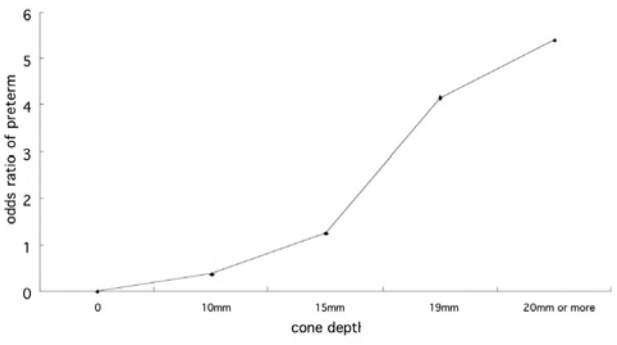

Figure 2. — Relation between cone depth and odds ratio of premature delivery.

In 48 patients of the LEEP group, 20 experienced vaginal delivery with normal birth process and without precipitate delivery and prolonged labour and 28 patients underwent cesarean section with indications including social factors in eight patients, intra-uterine asphyxia in two patients, breech position in two patients, cephalopelvic disproportion in one patient, gestation period complications in nine patients, and no per vagina trial labor after LEEP in six patients. In the 36 patients of the CKC group, 16 patients experienced vaginal delivery with normal birth process and without precipitate delivery and prolonged labor and 20 patients underwent cesarean section with indications including social factors in five patients, intra-uterine asphyxia in two patients, breech position in one patient, prolonged active phase in one patient, gestation period complications in six patients, and no per vagina trial labor after LEEP in five patients. In 68 women of the control group, 29 of them experienced vaginal delivery with normal birth process and without precipitated delivery and prolonged labor; 39 women underwent cesarean section with indications including social factors in 13 women, intra-uterine asphyxia in six women, breech position in two woman, horizontal position in one woman, cephalopelvic disproportion in two women, oligohydramnios in three women, persistent occipitoposterior position in one woman, and gestational complications in 11 women. There were no significant differences in the cesarean section rate between the three groups ($\chi^2 = 0.065$, $p = 0.968$).

In the LEEP group, the average birth weight was 3,350 g (range 1,450 - 3,850), the birth weight was less than 2,500 g in nine neonates, there was no macrosomia, and one minute Apgar score was more than 7 in all neonates. In the CKC group, the average birth weight was 2,950 g (range 1,050 - 3,800), the birth weight was less than 2,500 g in 15 neonates, there was no macrosomia, one minute Apgar score was less than 7 in two neonates, and in other neonates one minute Apgar score was more than 7. In the control group, the average birth weight was 3,450 g (range 650 - 4,200), the birth weight was less than 2,500 g in ten neonates, three neonates were macrosomia, a mid-trimester neonate died, one minute Apgar score was less than 7 in three neonates, and in other neonates one minute Apgar score was more than 7. The neonates with a birth weight of less than 2,500 g was significantly greater in the CKC group than in the control group ($\chi^2 = 7.952$, $p = 0.005$).

Discussion

CIN has become a common disease in women of childbearing age. Conization is often used to treat CIN and it mainly includes LEEP and CKC. Whether conization has adverse effects on pregnancy has been a concern of patients and doctors. With LEEP, high-frequency electric waves allow tissue to produce impedance, then oscillation produces high temperatures which leads to intracellular water evaporation, followed by cell rupture and tissue separation. LEEP is a common and effective method for treatment of CIN because it has some advantages including little radiation on surrounding tissue, little bleeding, simple procedures, minimal invasion, shorter operative time, and low cost. With CKC, cervical lesions were removed with a cryo-scalpel, the excisional extent and depth may be controlled, and there is no electrical damage on surrounding tissue. Connective tissue, smooth muscle, blood vessels, and elastic fibers comprise the cervix which plays an important role in pregnancy and delivery. Excessive tissue excision may lead to loose cervix or cervical incompetence which is likely to result in higher premature birth and abortion. Partial removal of cervical tissue may affect its function, especially on subsequent pregnancy [6]. In the present study, pregnant women who underwent colposcopic biopsy but did not receive any treatment, were considered the control to observe the effects of LEEP or CKC on pregnancy outcomes. The results in this study indicated that compared with the control group, premature delivery rate was significantly increased with OR of 2.455 (1.007 - 5.985), gestational week was significantly shorter, and neonates weighing less than 2,500 g were significantly increased; but there were no significant differences in uterine-incision delivery rate and neonatal asphyxia in the CKC group. A Chinese study has reported that CKC has no adverse effects on pregnancy [3]. This may be related to case number and inclusion criteria. The results in the present study are similar to those of most foreign studies

[6] regarding that CKC has adverse effects on pregnancy, such as increased premature birth risk and the rate of low birth weight infants. However, this study indicated that LEEP results were different from CKC. Compared with the control group, LEEP had no adverse effects on postoperative pregnancy. This may be related to less number of cases. Chinese studies in essence indicate that LEEP has no adverse effects on postoperative pregnancy [7], which is different from foreign studies [8-10]. Is this phenomenon perhaps related to race? The results suggest that LEEP may be safer than CKC and patients with CIN who desire to become pregnant should undergo LEEP as much as possible instead of CKC.

The authors further analyzed premature delivery-related factors after conization. Among lesion severity, and cone depth and area, premature delivery was strongly related to cone depth, which is consistent with the results reported by Noehr *et al*. [3, 6]. It was also found that less than 10 mm cone depth scarcely increased the risk of premature delivery; a 15 mm cone depth had a OR of 1.259, and more than 15 mm cone depth markedly increased OR. Grane [11] has described that less than 10 mm cone depth has no marked effects on pregnancy. Houlard *et al*. [12] have reported that with LEEP, when the excision depth is more than 20 mm, the incidence of postoperative cervical stenosis is significantly increased. Sadler *et al*. [13] have found that when the cone depth is more than or equal to 17 mm, the incidence of PROM is more than three times the incidence in the control group. However, there is also a report that cone depth is not related to premature delivery [14]. The results in the present study suggest that under the condition to ensure adequate removal of the lesion, the cone depth should be controlled within 15 mm, which may help to decrease the risk of premature delivery.

For LEEP or CKC, their effects on pregnancy also including postoperative cervical stenosis, infertility caused by postoperative pelvic infection, and ectopic pregnancy, remain to be further studied.

In summary, this study suggests that CKC may have certain adverse effects on postoperative pregnancy and pregnancy after LEEP is relatively safe; under the condition to ensure adequate removal of the lesion, the cone depth should be controlled within 15 mm, which may help to decrease the risk of premature delivery. The limitations of this study include less case number, shorter follow-up time, and more pregnancy-related factors. Large-scale studies will be performed in the future.

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Evaluation of osteopontin and CA125 in detection of epithelial ovarian carcinoma

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Summary

The objective of this study was to determine the potential of osteopontin (OPN) and OPN + CA125 (cancer antigen 125) combination in differential diagnosis of the ovarian cancers and non-malignant ovarian disease. Serum and plasma samples were obtained preoperatively from 79 women undergoing surgery for pelvic mass; 48 of them had ovarian carcinoma, and 31 had benign cyst. The samples were analyzed for the levels of OPN and CA125 (using ELISA and CMIA methods) and then compared with the final pathologic results. The median plasma level of OPN in patients with benign and malignant cysts was 356.33 ng/ml and 865.15 ng/ml, respectively ($p < 0.001$). Receiver operating characteristic (ROC) analysis for plasma OPN revealed the area under the curve (AUC) of 0.838. At the predefined specificity of 90%, OPN showed sensitivity of 62.5%, whereas the combination of OPN + CA125 reached 74.9% at the same specificity.

Key words: Ovarian cancer; Osteopontin; CA125; Tumor markers.

Introduction

It is well-known that an ovarian cyst may be diagnosed in a large number of women, even 20%, at some point in their lifetime. Accurate diagnostics aim to differentiate benign cysts from malignant ovarian tumors and provide adequate and fast treatment of patients. It is believed that 5% - 10% of women present for surgical interventions due to suspected ovarian neoplasm, out of which ovarian malignancy is verified in only 13% - 21% of these [1]. Conversely, if ovarian cancer (OC) is not detected at an early stage, the odds for successful treatment would be very low [2]. Most commonly used and currently best-studied tumor marker for diagnosis of ovarian neoplasms is cancer antigen 125 (CA125), but its utilization is limited by insufficient sensitivity and specificity.

Osteopontin (OPN) is another potential cancer biomarker. It is secretory glyco-phosphoprotein present in all body fluids and in the extracellular matrix. OPN is involved in cellular signaling pathways related to adhesion, cell migration, prevention of apoptosis, and neovascularization [3]. Its role in tumor genesis and progression of metastases has been confirmed.

This prospective study was designed to evaluate the significance of osteopontin in diagnostics of women presenting with suspected cystic pelvic mass and to summarize the combination of osteopontin and CA125 in detection of ovarian cancer.

Materials and Methods

The study included 79 subjects whose age ranged from 25 to 80 ($\bar{x} = 59.3 \pm 13.8$) years, and treated at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia, between January 2008 and December 2010 (Table 1). The study group included women with an ovarian cyst or pelvic mass, who were scheduled for surgery to ensure the correct diagnosis of possible epithelial ovarian cancer. Histopathological analysis, presented in Table 2, showed that, out of a total number of patients, 31 (39.2%) had benign cysts and 48 (60.8%) had epithelial ovarian cancer, [12 (15.2%) - Stage I, six (7.6%) - Stage II, 23 (29.1%) - Stage III and 7 (8.9%) - Stage IV].

Plasma OPN was measured by enzyme-linked immunosorbent assays (ELISA Kit), and serum CA125 using a chemiluminescent enzyme immunoassay (CMIA) test on the Architect i System.

Statistical methods used ANOVA for testing the differences between the benign and malignant group, histopathological subgroups of malignant group, and FIGO stages. The applied non-parametric tests were as follows: Kruskall-Wallis test and Mann-Whitney *U* test. Linear regression model was used to determine the relationship between the biomarkers. Receiver operator characteristic (ROC) curves were plotted for each biomarker in relation to benign and malignant tumors. SPSS Statistics software (ver. 15) was used for all statistical analyses. Statistical significance was set at value of $p < 0.05$.

Results

Biomarkers osteopontin and CA125 were determined in collected blood samples and it was found that the values of both biomarkers were significantly higher in patients with malignant cysts in comparison to the benign group ($p < 0.001$). There was also a significant difference between the median value of these two markers in the

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Table 1. — *Characteristics of patients (n = 79).*

	No. of patients	%	*p* value
Diagnostic category			
Benign	31	39.2%	0.056
Malignant	48	60.8%	
Age at diagnosis (years)			
< 30	4	5.06%	< 0.001
31 - 40	5	6.33%	
41 - 50	11	13.92%	
51 - 60	17	21.52%	
61 - 70	26	32.92%	
> 70	16	20.25%	

Table 2. — *Histology of cancer.*

Cancer Stage	FIGO I	FIGO II	FIGO III	FIGO IV	Total
Undifferentiated			1		1
Mucous	4		3	2	9
Serous	4	3	11	4	22
Mixed	2		1		3
Clear cell		1	1		2
Endometrioid	2	2	6		10
Brenner tumor				1	1
Total	12	6	23	7	48

Table 3. — *Descriptive statistics of serum OPN and CA 125 in benign and cancer patients.*

Biomarker	Groups	n	Median	Min	Max	*p* value
OPN	Benign	31	356.33	56.70	1,000.80	$p < 0.001$
	EOC	48	865.15	89.07	4,512.30	
	Stage I / II	18	415.25	89.07	1,220.00	$p < 0.001$
	Stage III / IV	30	1,445.50	379.75	4,512.30	
CA125	Benign	31	30.10	5.90	141.80	$p < 0.001$
	EOC	48	997.85	15.40	2,972.30	
	Stage I / II	18	94.85	15.40	2,600.32	$p < 0.001$
	Stage III / IV	30	1,447.96	125.40	2,972.30	

early phase of disease (Stage I/II) and late phase (III/IV) of epithelial ovarian cancer (EOC) ($p < 0.001$), (Figure 1). Table 3 illustrates the descriptive statistics of OPN and CA125 in blood of patients with the benign cysts, as well as patients with various stages of ovarian cancer.

Mann-Whitney test confirmed that there was no significant difference of OPN values between different histological types of tumors (Figure 2).

Linear regression analysis showed that there was a low correlation coefficient between the OPN and CA125 values in the group with malignant cysts ($r^2 = 0.1489$), and slightly higher correlation coefficient between the OPN and CA125 values in the group with benign cysts ($r^2 = 0.1522$) (Figure 3). The low correlation coefficient indicated that a combination of these two biomarkers would improve their individual abilities for cancer detection.

ROC analysis of preoperative plasma OPN and CA125 for all patients are presented in Figure 4. The area under the curve (AUC) for OPN was slightly inferior [AUC = 83.8%; 95% CI (75 - 92.5%)] to area under the CA125 [AUC = 90.3%; 95% CI (83.7 - 96.8%). At the predefined specificity of 90%, OPN and CA125 showed sensitivity of 62.5% and 72.6%, respectively, whereas the combination of OPN + CA125 reached 74.9% at the same specificity. Arbitrary cut-off level for plasma osteopontin was 650 ng/ml.

Discussion

Ovarian cancer is one of the most common reproductive cancers and has the highest mortality rate among gynecologic cancers. Most of ovarian cancer diagnoses occur in the late stages of the disease and five-year survival rates fall below 20%. To overcome the significant mortality associated with ovarian cancer, research on the clinical significance of new sensitive and specific biomarkers/panels of biomarkers are still very important.

In this paper, the authors reported that plasma OPN could augment CA125 detection, providing higher sensitivity and specificity in predicting ovarian cancer. With a sensitivity level of 62.5% alone (specificity 90%) OPN may have a lower potential than CA125 to accurately detect the presence of ovarian cancer. High sensitivity was achieved, reaching 74.9% (specificity 90%) when OPN was combined with CA125 in a biomarker screening panel. The obtained results show better characteristics of OPN as a tumor marker from the one that was given from Nakae *et al*. [4]. Regarding the present samples, there was no significant difference of plasma OPN concentration in different histological types of tumors, suggesting that all histological EOC types have increased plasma level of OPN. This is in agreement with the findings of Tiniakos *et al*. [5]. However, the authors proved that plasma OPN was significantly elevated during advanced stages of the disease, but there was also border significance between benign patients and early stage of disease. All these results suggest the potential use of plasma OPN and CA125 serum values for ovarian cancer diagnostic.

Ovarian cancer is known as the "silent killer", with very weak, nonspecific symptoms. For this reason, using a non-invasive approach, such as tumor markers for detection of the disease, is still very attractive. A number of proteins present in either blood or urine have been identified as specific markers for epithelial ovarian cancer [6, 7]. However, no single protein has provided adequate sensitivity and specificity for distinguishing malignant from benign pelvic masses. Some recent studies described panels of biomarkers that beside OPN had four [8], five [9], or more biomarkers [10] with high sensitivity and specificity for ovarian cancer detection. This present study suggests that measuring of preoperative plasma OPN and CA125 could provide a cost-effective and sensitive test in triage women with pelvic mass and therefore, reduce disease-associated mortality.

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Fig. 1

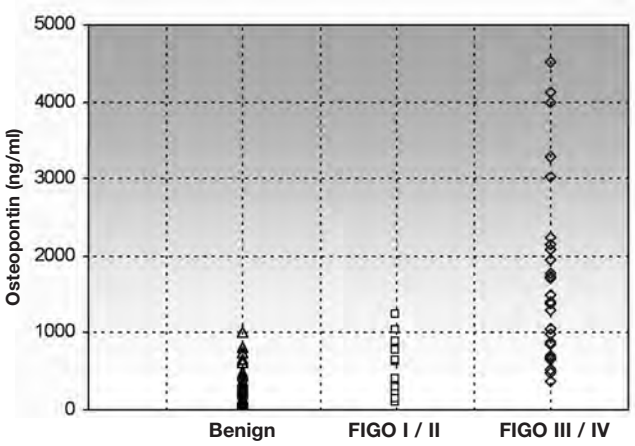

Fig. 2

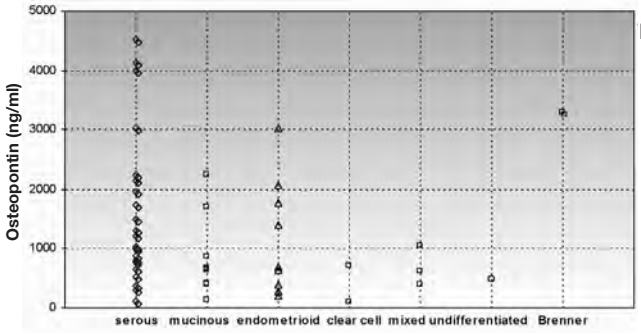

Fig. 3

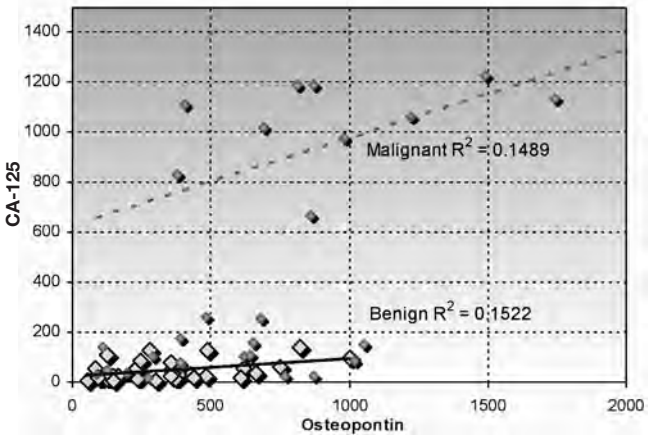

Fig. 4

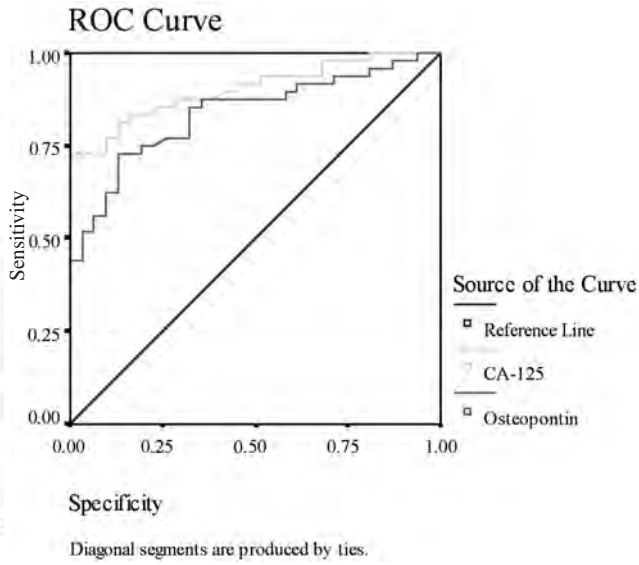

Figure 1. — Preoperative levels of osteopontin in patients with benign and malignant ovarian mass.
Figure 2. — Osteopontin levels in patients within histology of cancer.
Figure 3. — Linear regression curves.
Figure 4. — ROC analysis of OPN and CA125 for patients with the benign and malignant cysts.

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Case Reports

Primary peritoneal low-grade serous carcinoma forming a mass in the colon mimicking a colonic primary carcinoma: a case report

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Summary

Primary carcinomas of Müllerian origin involving the colon is not an uncommon phenomenon, with most cases reportedly associated with endometriosis. On the other hand, a primary peritoneal low-grade serous carcinoma presenting as a dominant mass in the colon and causing clinical symptoms mimicking a primary colonic carcinoma has not been reported in the literature to the best of the authors' knowledge. A case of a 66-year-old female patient who presented clinically with rectal bleeding and a rectosigmoid mass is described. The final histologic examination revealed a peritoneal low-grade serous carcinoma forming a dominant mass in the rectosigmoid colon. Of particular interest was a microscopic spectrum of serous epithelial proliferation in the peritoneal cavity and lymph nodes with morphologic features reminiscent of non-invasive and invasive implants in ovarian borderline serous tumors, which most likely denoted the precursors of the tumor in the colon.

Key words: Peritoneal; Low-grade serous carcinoma; Colonic mass.

Introduction

The colon is not uncommonly involved by neoplasms of Müllerian differentiation, with most of the cases secondary to direct invasion or indirect dissemination by a tumor from the adjacent gynecologic organs. There are however rare cases of primary tumors of Müllerian differentiation arising in the colon [1, 2], with the vast majority of these cases associated with foci of endometriosis located in the colonic wall. In the current case report, the authors describe an extraordinary case of a primary peritoneal low-grade serous carcinoma presenting with clinical symptoms of a colorectal carcinoma. The dominant mass was located in the rectosigmoid colon and was associated with a microscopic spectrum of peritoneal and nodal serous epithelial proliferation exhibiting histologic features reminiscent of non-invasive and invasive implants of the more common ovarian borderline serous tumors. To the best of the authors' knowledge, this case is the first report of a primary peritoneal low-grade serous carcinoma predominantly involving the colon and clinically presenting with rectal bleeding as the first manifestation of the disease.

Case Report

The patient, was a 66-year-old Caucasian female who presented at an outside hospital with a history of rectal bleeding. The outside colonoscopy revealed a friable mass in the rectosigmoid colon. Biopsy of the rectosigmoid mass demonstrated an adenocarcinoma with histologic and immunohistochemical features suggestive of an ovarian carcinoma involving the colon. The patient was referred to the hospital in November 2010 and underwent exploratory laparotomy with resection of the rectosigmoid colon, left salpingo-oophorectomy, omentectomy, appendectomy, pelvic and aortic lymphadenectomy, and peritoneal staging biopsies. Intraoperative findings indicated a mass in the rectosigmoid colon and possibly small-volume disease around the appendix and on the ascending colon mesentery. No additional evidence of disease was visible in the abdominal peritoneal cavity. The omentum and left ovary were unremarkable at the time of the surgery. After surgery, the patient underwent a chemotherapy protocol for serous carcinoma including six cycles of carboplatin and taxol. At the last follow-up in January 2012, the patients was alive and did not show any clinical or radiologic evidence of tumor recurrences.

On gross inspection, the rectosigmoid colon demonstrated a 5 x 4 x 2 cm mass involving all layers of the colon and invading the pericolic adipose tissue. The left ovary and Fallopian tube, omentum, appendix, and the resected lymph nodes were grossly unremarkable.

The colonic tumor was characterized by clusters and nests of relatively monotonous and eosinophilic cuboidal cells with mild to moderate cytologic atypia arranged in papillary architecture (Figures 1A and 1B). Numerous psammoma bodies were identified in the tumor. The neoplastic nests and clusters were surrounded by retraction clefts. The mitotic activity varied between five to ten mitoses per ten high-power fields. The histologic findings were consistent with those of a low-grade serous carcinoma based on the two-tier grading system for serous carcinomas proposed by Malpica *et al*. [3]. Nests of non-invasive low-grade serous proliferation were identified on the serosa of the colon, adjacent to the tumor (Figure 1C). Although grossly unremarkable, histologic examination of the omentum demonstrated a spectrum of serous epithelial proliferation. The vast majority of the serous proliferation displayed morphologic features reminiscent of non-invasive implants in the context of borderline serous tumors of the ovary (Figure 2A). Some foci measured up to 0.3 cm and exhibited desmoplastic reaction, an increased ratio of epithelial proliferation to stroma, and a deep invasion into the omentum consistent with invasive implant-like foci of serous neoplasms (Figure 2B). There were several positive pericolic lymph nodes that contained nests and clusters of serous neoplastic cells associated with a desmoplastic reaction (Figure 2C). On the other hand, two positive inguinal lymph nodes exhibited only rare nests of serous neoplastic cells adja-

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Fig. 1A

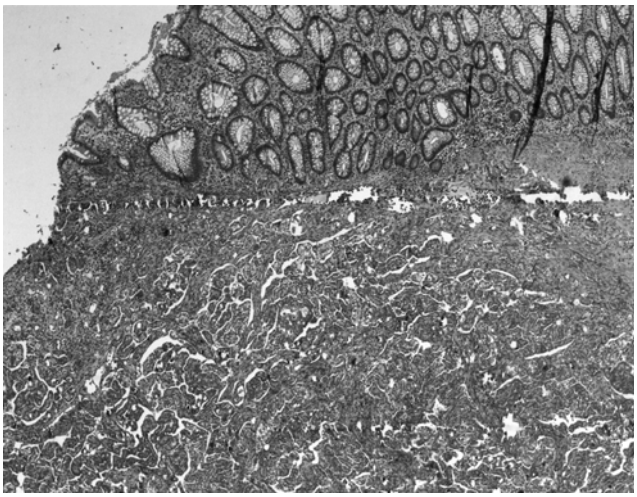

Fig. 1B

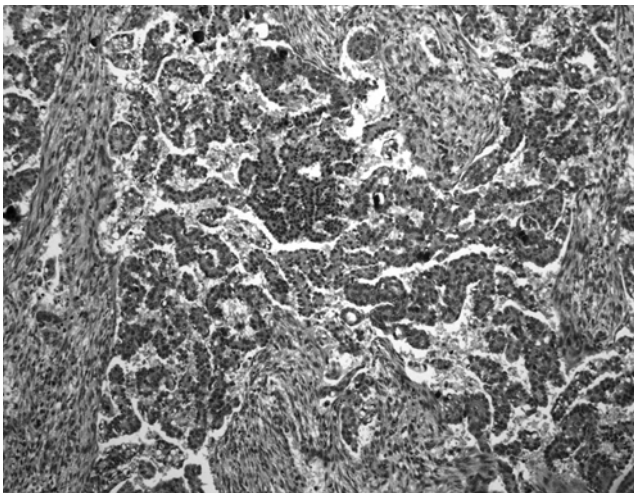

Fig. 1C

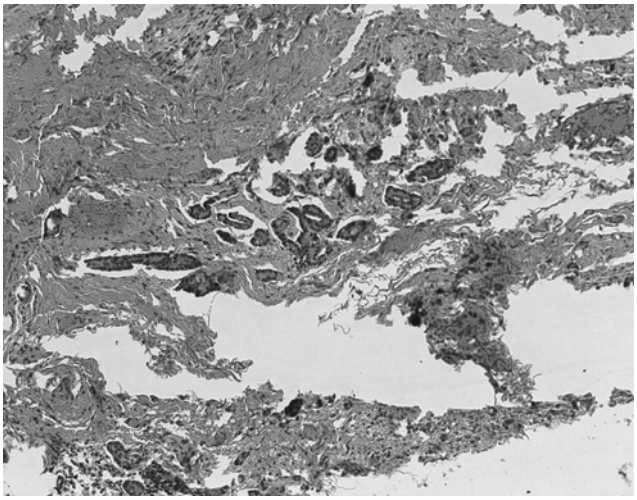

Figure 1. — Classic histologic features of a low-grade serous carcinoma involving the colonic wall (A and B) and a microscopic focus of non-invasive serous proliferation on the adjacent serosa of the colon (C, x100).

cent to benign epithelial inclusion cysts/endosalpingiosis without any desmoplastic reactions (Figure 2D). Immunohistochemical studies revealed that the tumor cells were strongly positive for cytokeratin 7 (Figure 3A), Estrogen receptor, and WT-1 (Figure 3B). Although the neoplastic cells were immunoreactive for cytokeratin 20 (Figure 3C), they were negative for Cdx-2. The immunohistochemical results further supported a serous carcinoma involving the colon.

Discussion

Tumors of Müllerian origin involving the gastrointestinal (GI) tract, particularly the colon, is not an infrequent occurrence. These tumors involve the colon in two major pathways. The most common pathway is direct or indirect spreading of tumors from an adjacent gynecologic organs or peritoneal cavity to the colon. Less commonly are primary neoplasms of Müllerian origin that arise from foci of endometriosis or endosalpingiosis located in the colonic wall [1, 2]. However, a primary peritoneal low-grade serous carcinoma presenting as a colonic tumor without any visible extracolonic mass is until now an unreported phenomenon.

With the advancements in colonic endoscopy, more tumors of Müllerian differentiation are diagnosed preoperatively as in this case. Because the preoperative biopsy revealed a possible ovarian carcinoma involving the colon, it was suspected at the time of the surgery that the tumor was most likely an endometriosis-related colonic carcinoma. Unexpectedly, the histologic examination revealed a low-grade serous carcinoma in the colon without any other macroscopically obvious mass in the ovaries and omentum. The omentum, however, demonstrated a spectrum of serous epithelial proliferation with morphologic features reminiscent of non-invasive and invasive implants commonly described in borderline serous tumors of the ovary. In the absence of a dominant mass in the omentum and ovaries, it is highly unlikely that the serous carcinoma in the colon represented a metastasis from an adjacent gynecologic organ. Extensive histologic evaluation of the colon did not reveal any foci of endometriosis, which was not unexpected since serous carcinomas do not belong to the endometriosis-related carcinomas. As a matter of fact, in a recent study of endometriosis-associated intestinal tumors with a review of the literature, no case of low-grade serous carcinoma was documented in the series [1]. Therefore, the low-grade serous carcinoma in the colon in the current case report was most likely related to the background of serous

Fig. 2A

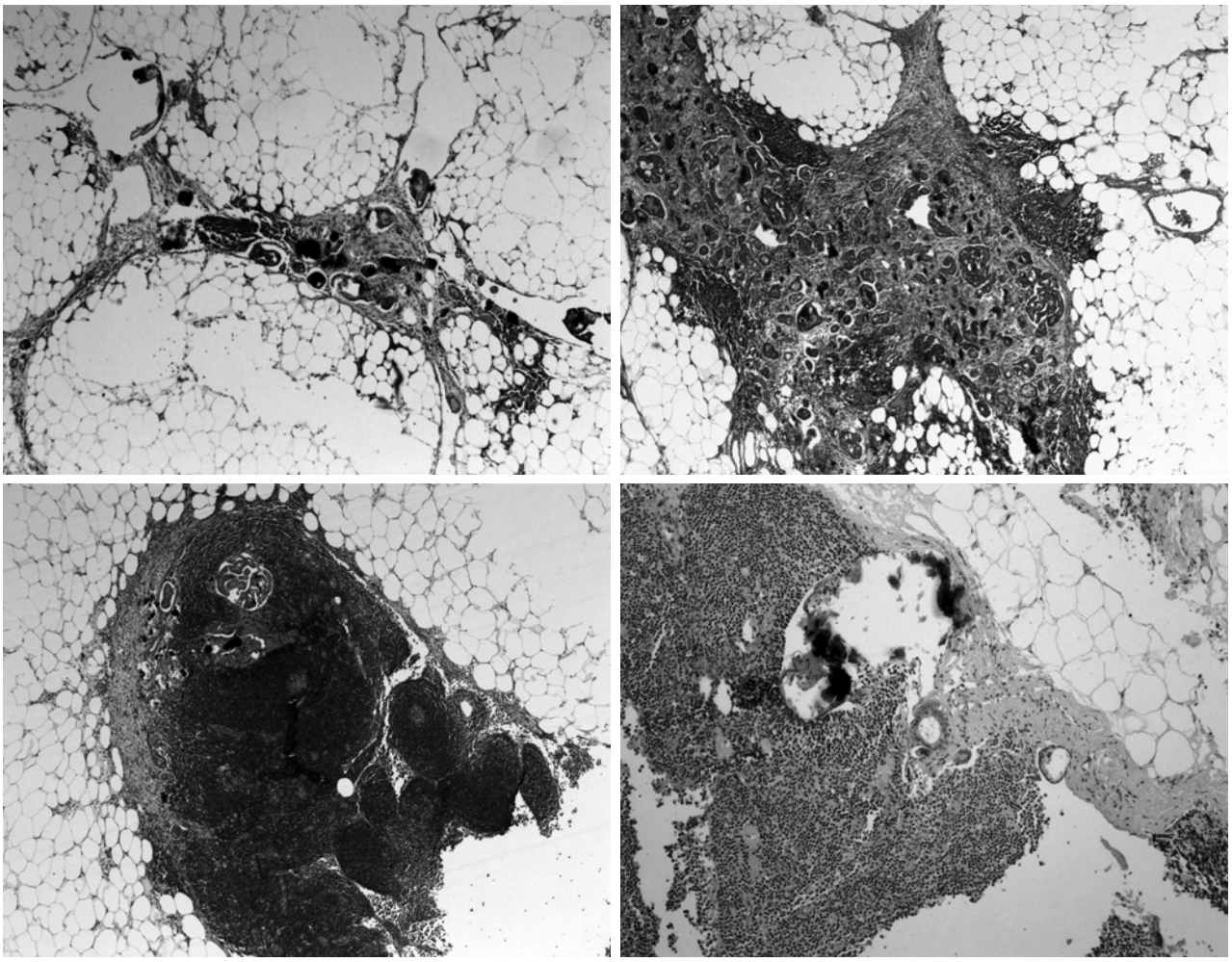

Fig. 2B

Fig. 2C

Fig. 2D

Figure 2. — A microscopic non-invasive (A) and a 0.3 cm invasive (B) focus of implant-like serous proliferation in the omentum. Nests of serous neoplastic cells associated with a desmoplastic reaction in a pericolic lymph node (C). An inguinal lymph node showing a small focus of endosalpingiosis and clusters of serous cell proliferation (D).

proliferation in the omentum and lymph nodes. It is reasonable to postulate that the serous carcinoma in the colon represented the final tumor progression step of the multifocal serous proliferative process in the abdominal cavity, which probably began as a non-invasive implant-like foci, with one focus eventually transformed into the low-grade serous carcinoma of the colon. Based on these considerations, the tumor in the colon is best classified as a primary peritoneal low-grade serous carcinoma. Also of interest was the discrepancy in the morphology of the microscopic foci of serous proliferation in the lymph nodes along the colon and those in the inguinal lymph nodes. Whereas those in the lymph nodes along the colon were associated with a desmoplastic reaction and therefore most likely represented true metastases, the serous nests in the inguinal lymph node appeared to arise from benign epithelial inclusion cysts/endosalpingiosis and hence were more likely independent foci of serous proliferation. In agreement with this idea is a recent study from the MD Anderson Cancer Center, which demonstrated that "in up to a third of patients with ovarian serous tumors of low malignant potential and lymph node involvement, nodal foci of serous tumor of low malignant potential may derive independently from nodal endosalpingiosis due to a field effect" [4]. The dual immunoreactivity of the neoplastic cells for both cytokeratin 7 and cytokeratin 20 in the current case report is unusual but has been reported in the literature. Groisman *et al*. [5] detected weak to strong staining for cytokeratin 20 in up to 67% of their cohort of serous carcinomas.

A recent study of 13 cases of "carcinoma of Müllerian origin presenting as colorectal cancer" revealed a spectrum of neoplasms including endometrioid carcinoma, mixed papillary serous and endometrioid carcinoma, undifferentiated carcinoma, and malignant mixed Müllerian tumor [1]. Endometriosis was identified in nine out of 13 cases. One case of malignant mixed Müllerian tumor was associated with endosalpingiosis. Therefore, the authors preferred the term "carcinoma of Müllerian origin" instead of "endometriosis associated carcinoma" for this type of neoplasms in the colon. Based on that conceptual model, this current case report of a peritoneal

Fig. 3A

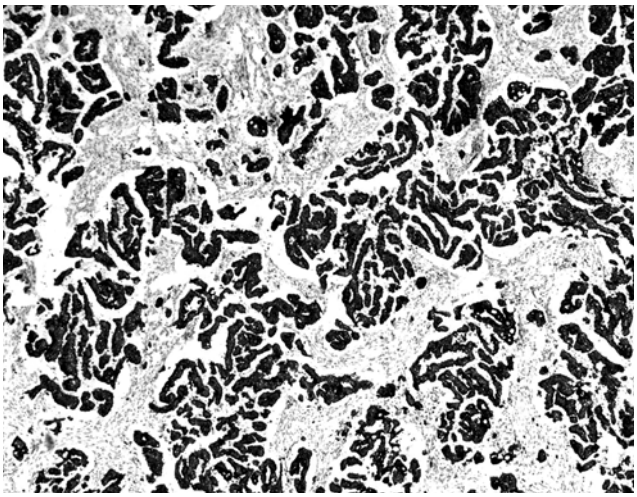

Fig. 3B

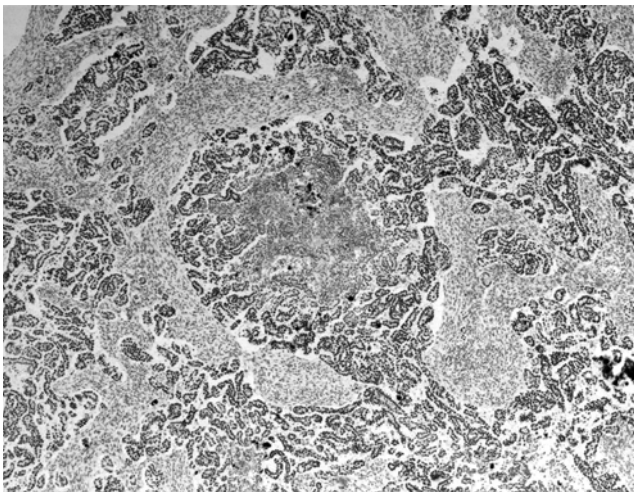

Fig. 3C

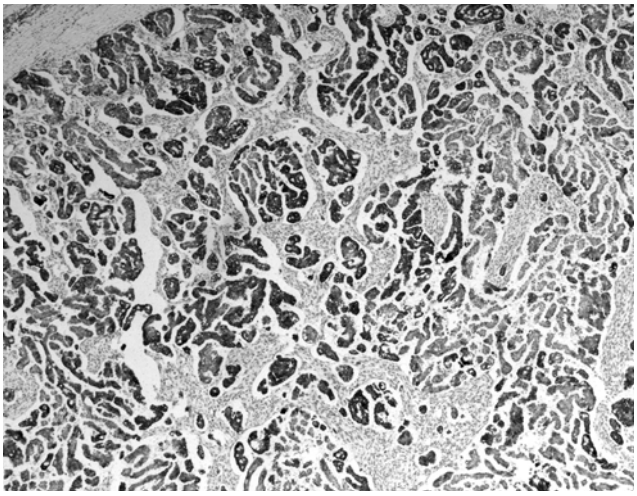

Figure 3. — Immunohistochemistry showing that the neoplastic cells are positive for cytokeratin 7 (A), Cytokeratin 20 (B), and WT-1 (C).

low-grade serous carcinoma primarily involving the colon broadens the spectrum of "carcinoma of Müllerian origin presenting as colorectal cancer".

Because the pathologic staging, treatment, and prognosis of a primary carcinoma of Müllerian differentiation arising in the colon differ significantly from those of a conventional primary colonic adenocarcinoma, or a metastatic carcinoma of Müllerian origin from an adjacent gynecologic organ to the colon, an accurate diagnosis and staging of a tumor of Müllerian origin involving the colon are critical for clinical managements. From a therapeutic standpoint, this tumor should be considered as a primary peritoneal low-grade serous carcinoma with an unusual clinical manifestation as a colonic tumor instead of the more common abdominopelvic mass and call for a proper chemotherapic protocol.

Conclusion

In summary, the authors report a case of a primary peritoneal low-grade serous carcinoma with clinical presentation mimicking a primary colonic cancer. The case underscores the importance of the awareness of such a rare phenomenon, not only from a diagnostic standpoint, but also in terms of therapeutic considerations.

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Complete remission of recurrent and refractory ovarian cancers using weekly administration of bevacizumab and gemcitabine/oxaliplatin: report of two cases

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Summary

Background: A combination therapy with gemcitabine and oxaliplatin (GEMOX) yielded a moderate activity in platinum-resistant ovarian cancers; however, frequent severe toxicities, such as thrombocytopenia and neurotoxicity, were observed. A certain modification of schedule might therefore facilitate the clinical application of the regimen. The authors report two cases that achieved complete response to a weekly administration of bevacizumab and GEMOX. *Materials and Methods:* Two patients with platinum-resistant recurrent ovarian cancers received a weekly regimen of GEMOX with bevacizumab: 2 mg/kg of bevacizumab, 300 mg/m^2 of gemcitabine, and 30 mg/m^2 of oxaliplatin, three weeks on and one week off, Q4 weeks. Complete remission was observed after three to four courses of therapy. Hematologic and non-hematologic toxicities more than grade 2 were not observed during chemotherapy. The patients are now without tumor progression more than 12 months after initiation of therapy. *Conclusion:* Weekly administration of bevacizumab and GEMOX had potential activity in recurrent and refractory ovarian carcinomas. These findings warrant necessity of further trial in such clinical settings.

Key words: Ovarian cancer; Recurrence; Weekly therapy; Bevacizumab; Gemcitabine/oxaliplatin.

Introduction

Although epithelial ovarian cancers are a chemo-sensitive disease that respond to initial platinum/paclitaxel chemotherapy with a high response rate, more than half of patients with advanced disease develop recurrence and require further therapy [1]. For the treatment of recurrent or refractory ovarian cancers, a single agent such as gemcitabine, pegylated doxorubicin, or topotecan is administered as a salvage treatment [2, 3]. However, the response is limited with median progression-free survival less than 12 months.

A combination therapy with gemcitabine and oxaliplatin (GEMOX) has been evaluated for recurrent ovarian cancers in several phase II studies. Overall response rates ranged from 20% to 37% [4], however, in patients with platinum-resistant disease, response was observed in less than ten percent of the cases. Additionally, frequent severe toxicities, such as thrombocytopenia and neurotoxicity, have been reported. Another phase II study of a combination therapy with bevacizumab (10 mg/kg), gemcitabine (1,000 mg/m^2), and oxaliplatin (65 mg/m^2) on days 1 and 15 in a 28-day cycle showed an extremely high response rate of 68.5% for platinum-sensitive ovarian cancers [5]. The response rate was approximately two times higher than previous reported rates with GEMOX. Thus, the authors attempted a weekly-based continuous regimen of bevacizumab combined with GEMOX (B-GEMOX) for heavily pretreated and refractory ovarian cancers. The present reports two cases with recurrent and refractory ovarian cancers that achieved complete remission by weekly B-GEMOX.

Case Report

Case 1

Case 1 is a 71-year-old patient with Stage IIIC ovarian serous cystadenocarcinoma who was referred to the Clinic because of refractory and recurrent disease located in the pelvis, para-aortic lymph nodes, and lungs (Figures 1A-C). Five years ago, the patient received two cycles of neoadjuvant chemotherapy with weekly paclitaxel and carboplatin (wTC) followed by primary debulking surgery. Subsequently, she received six cycles of wTC, however, recurrent disease was seen in the pelvis three years ago. The patient underwent debulking surgery for the pelvic mass, and since then all regimens she received did not show a response: ten cycles of combination with irinotecan and paclitaxel, four cycles of pegylated liposomal doxorubicin, and three cycles of irinotecan and cisplatin. After consultation in the Clinic, the patient received the following weekly regimen with B-GEMOX therapy: 2 mg/kg of bevacizumab, 300 mg/m^2 of gemcitabine, and 30 mg/m^2 of oxaliplatin, three weeks on and one week off, Q4 weeks. Grade 1 toxicities of neutropenia, nausea, fatigue, and skin pain were observed, but there were no toxicities greater than grade 2. Complete response (CR) was observed after four cycles of B-GEMOX (Figures 1D-F). Massive ascites and bilateral pleural effusion also completely disappeared. Eastern Cooperative Oncology Group (ECOG) physical status was improved as ascites decreased: two at the initiation of B-GEMOX, and none after three cycles of B-GEMOX. Additional four cycles were administered with 16 months from the initiation of B-GEMOX and the patient is now without tumor progression.

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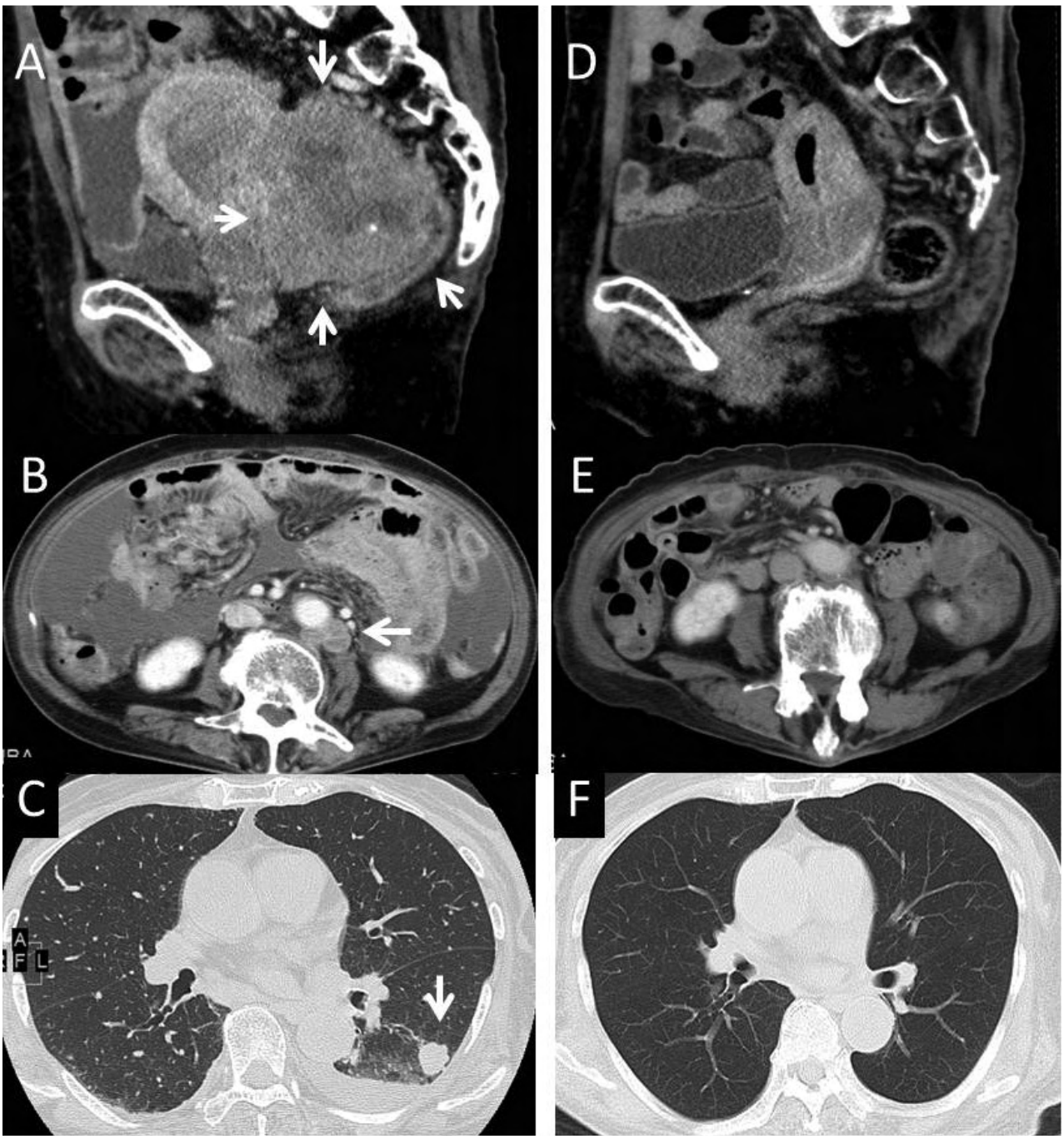

Figure 1. — CT images of recurrent and refractory ovarian cancers. A, B, and C: recurrent and refractory tumors before combination therapy with bevacizumab and GEMOX (arrow). D, E, and F: all refractory tumors, massive ascites, and pleural effusion were not detected by CT images after four cycles of the therapy, achieving CR. Images D, E, and F correspond with images A, B, and C, respectively.

Case 2

Case 2 is a 53-year-old patient with Stage IIIC ovarian serous cystadenocarcinoma who was referred to the Clinic because of refractory peritoneal dissemination. For the treatment of recurrent tumors, a combination using paclitaxel and carboplatin was not effective, and another regimen with ifosfamide, epirubicin, and cisplatin also failed. At consultation in the Clinic, positron emission tomography-computed tomography (PET-CT) images showed multiple peritoneal disseminations with a moderate amount of ascites. She received three cycles of weekly B-GEMOX at an equivalent dose as Case 1 and achieved CR by PET-CT images. The ascites also disap-

Table 1. — *Response of combination therapy with gemcitabine and oxaliplatin for platinum-resistant ovarian cancers*.

Authors Dose of drugs (mg/m^2)	Response rate CR	 PR	Major grade 3/4 toxicities
Raspagliesi *et al*. 2004 G: 1,000 d. 1.8 O: 130 d. 8 (every 3 w)	0%	0%	40% neutropenia; 70% thrombocytopenia; 5% nausea/vomiting, liver dysfunction
Germano *et al*. 2007 G: 1,000 d. 1 O: 100 d. 2 (every 2 w)	10%	14%	37% neutropenia; 19% anemia; 4% nausea/vomiting
Kakykaki *et al*. 2008 G: 1500 d. 1.8 O: 130 d. 8 (every 3 w)	4%	15%	42% neutropenia; 10% anemia; 24% thrombocytopenia; 12% nausea/vomiting; 12% asthenia 2% neurotoxicity, allergy, edema
Harnett *et al*. 2007 G: 1,000 d. 1.8 O: 130 d. 8 (every 3 w)	0%	9%	24% neutropenia; 11% thrombocytopenia 16% nausea; 9% neuropathy; 7% dyspnea
Ray-Coquard *et al*. 2009 G: 1,000 d. 1.8 O: 100 d. 1 (every 3 w)	CR + PR = 38%		51% neutropenia; 26% thrombocytopenia 12% anemia; 7% nausea/vomiting; 8% asthenia
Horowitz *et al*. 2011 G: 1,000 d. 1.5 O: 65 d. 1.5 B: 10 mg/kg d. 1 (every 4 w)	NA	NA	26% neutropenia; 11% nausea/vomiting; 16% fatigue, neuropathy; 5% pulmonary embolism, hypertention, liver dysfunction

CR = complete response; PR = partial response; G = Gemcitabine; O = Oxaliplatin; B = Bevacizumab; NA = not available; D = day; w = weeks.

peared. Grade 1 toxicities of nasal bleeding, nausea, and dysgeusia were observed, however, there were no other severe toxicities. The patient received three more cycles of the therapy. Twelve months from the initiation of B-GEMOX, the patient is now with no sign of tumor progression.

Discussion

Response and major toxicities of combination therapy using gemcitabine and oxaliplatin (GEMOX) for platinum-resistant ovarian cancers are summarized in Table 1. The rate of complete response ranged from 0% to 10%, suggesting that the efficacy of GEMOX was limited as several anti-cancer agents used for platinum-resistant ovarian cancers [6-9]. Grade 3 and 4 toxicities were frequently observed in the patients treated with GEMOX: neutropenia, thrombocytopenia, neuropathy, asthenia, and so on (Table 1). The only phase II study evaluating the efficacy of combination with bevacizumab and GEMOX (B-GEMOX) yielded a response rate of 68.5%, however, the patients eligible for this trial were only platinum-sensitive relapsed ovarian cancers [5]. The rates of severe toxicities, such as neutropenia and thrombocytopenia, were lower in the Horowitz study (oxaliplatin = 65 mg/m^2, biweekly) as compared with other studies (oxaliplatin = 100-130 mg/m^2, every two to three weeks), suggesting that both dose and schedule of oxaliplatin were key factors for toxicities. The two patients presented were heavily treated with more than three regimens; the authors selected a weekly regimen to reduce severe toxicities, and doses of three drugs were almost half of those used in the Horowitz study: 2 mg/kg of bevacizumab, 300 mg/m^2 of gemcitabine, and 30 mg/m^2 of oxaliplatin, three weeks on and one week off, Q4 weeks. The present cases showed toxicities of grade 1 only: neutropenia, fatigue, skin pain, nasal bleeding, and dysgeusia in one case and nausea/vomiting in both cases. The weekly regimen of B-GEMOX showed excellent tolerability with less toxicity.

The present two cases had recurrent disease that was platinum-resistant and refractory to previous chemotherapy. CR was obtained after three to four cycles of B-GEMOX, and ascites with or without pleural effusion completely disappeared. In addition to complete response observed in recurrent tumors, reduction of ascites improved the quality of life, especially in patients with recurrent ovarian cancers. Additive effects of bevacizumab might be explained by the evidence that a combination of bevacizumab and a low-dose cytotoxic regimen blocks vascular repair and survival, enhancing the effects of cytotoxic drugs [10]. Also, reduction of ascites was explained by inhibition of vascular endothelial growth factor (VEGF) pathway, as ascites and plasma samples of ovarian cancers showed significant up-regulation of VEGF. [11]. Bevacizumab, a humanized recombinant antibody binding to VEGF, may have the potential to suspend the ascites production resulting from peritoneal dissemination in solid cancers including ovarian cancers [12].

Conclusion

Weekly administration of bevacizumab and GEMOX had potential activity in recurrent and refractory ovarian carcinomas. These findings warrant necessity of further trial in such clinical settings.

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Uterine malignant mixed Müllerian tumor after adjuvant tamoxifen treatment for breast cancer

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Summary

Background: Uterine malignant mixed Müllerian tumor (MMMT), also known as carcinosarcoma, is a biphasic tumor of the female genital tract and demonstrates both malignant epithelial (carcinoma) and mesenchymal (sarcoma) components. The authors present two cases of uterine MMMT after adjuvant tamoxifen (TAM) treatment for breast cancer and a review of the current literature. *Cases:* The patients presented with a complaint of abnormal uterine bleeding. They both had a history of breast cancer Stage IIB previously treated with modified radical mastectomy, at 51 and 78 months, respectively. They also had history of tamoxifen treatment 20 mg daily for seven and 73 months respectively. They underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy. Histopathology revealed a uterine MMMT. Postoperatively, they received adjuvant chemotherapy and radiotherapy. One of the patients died 26 months after initial surgery due to uterine MMMT. *Conclusion:* Uterine MMMT is a rare, highly-aggressive, and rapidly-progressing tumor associated with a poor prognosis. Postmenopausal patients, with prolonged adjuvant TAM treatment for breast cancer, are at increased risk for the development of uterine MMMT.

Key words: Uterine malignant mixed Müllerian tumor; Uterine carcinosarcoma; Tamoxifen; Treatment; Prognosis.

Introduction

Uterine malignant mixed Müllerian tumor (MMMT), also known as carcinosarcoma, is a biphasic tumor of the female genital tract and demonstrates both malignant epithelial (carcinoma) and mesenchymal (sarcoma) components [1, 2]. It is a very rare disease, accounting for 4.3% of all uterine malignant tumors [3]. Also, it has a worldwide annual incidence between 0.5 and 3.3 cases per 100.000 women [4, 5].

It usually occurs in postmenopausal women, although younger women may be affected. The median age at diagnosis of uterine MMMT is 62 years [6]. Uterine MMMT and endometrial cancer share a similar risk factor profile [7]. Risk factors for the development of uterine MMMT are: obesity, nulliparity, exposure to exogenous estrogens, and pelvic radiation [2-7].

The aim of this study was to describe the clinical characteristics, management, and prognosis of two patients with uterine MMMT after adjuvant tamoxifen (TAM) treatment for breast cancer that were diagnosed and treated in the Department and a review of the current literature.

Case Reports

Case 1

The patient, a 79-year-old gravida 2, para 2, postmenopausal Greek woman, presented with a complaint of abnormal uterine bleeding. She had a history of breast cancer Stage IIB previously treated with modified radical mastectomy 51 months ago. Postoperatively, she received adjuvant chemotherapy and radiotherapy. She also received 20 mg TAM daily for seven months. Her family history revealed no evidence of cancer among the first-degree relatives.

A gynecologic examination did not reveal any abnormal findings. There were no palpable inguinal lymph nodes and the rest of pelvic examination was also normal.

Preoperative computer tomography (CT) of the abdomen and pelvis, and abdominal ultrasound (U/S) revealed irregular endometrial thickening. A CT of the chest, chest X-ray, intravenous pyelography (IVP), colonoscopy, and urethrocystoscopy were normal. Dilation and curettage revealed uterine malignancy. Preoperative CA-125 was elevated at 120 U/ml.

During exploratory laparotomy, the patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy.

Histopathology revealed uterine MMMT. The epithelial component of uterine MMMT was adenocarcinoma and the mesenchymal component was leiomyosarcoma (Figures 1 and 2). The uterine tumor did not invade the myometrium. The ovaries and omentum were normal. The peritoneal washing smear was positive for malignant cells. The final diagnosis was Stage IA uterine MMMT according to FIGO staging system 2009 [8, 9].

The patient underwent postoperative adjuvant chemotherapy. She received six courses of carboplatin (400 mg/m^2). She also received postoperatively 5,000 cGy of external and 2,000 cGy of intravaginal radiotherapy. However, 22 months after initial surgery for uterine MMMT, the patient presented with a complaint of abnormal vaginal bleeding. A gynecologic examination revealed local recurrence of the disease with in the vaginal vault with dimensions of 5 x 3.5 x 2 cm. A CT of the abdomen and pelvis, and histopathology, re-confirmed the clinical diagnosis.

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Fig. 1

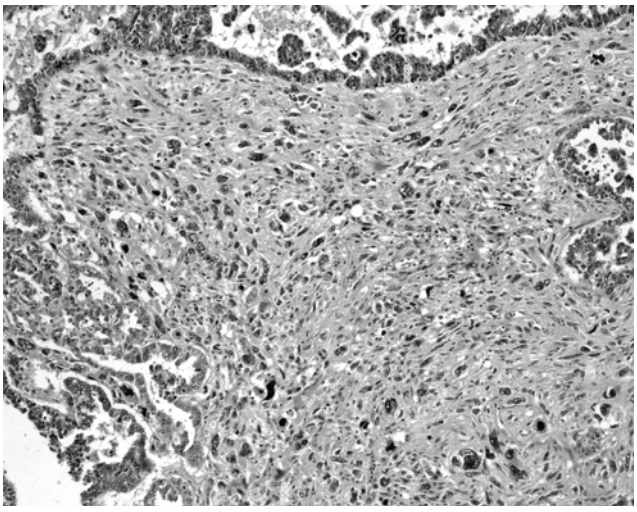

Fig. 2

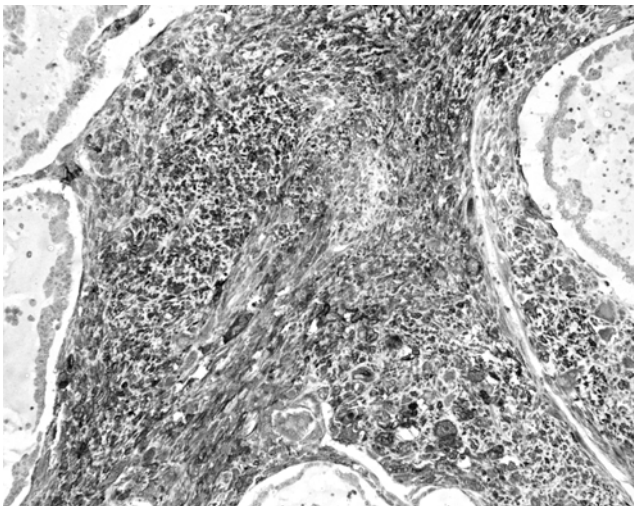

Fig. 3

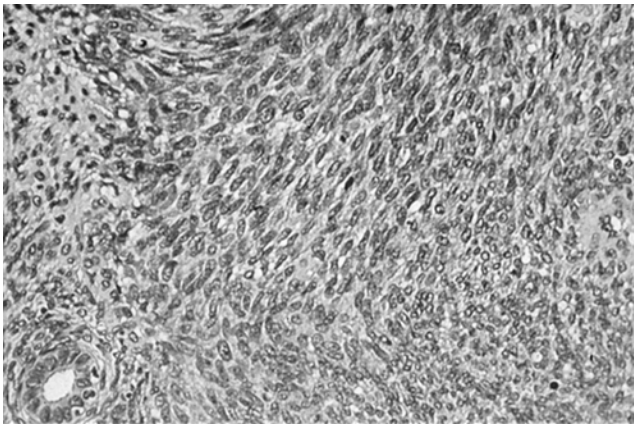

Fig. 4

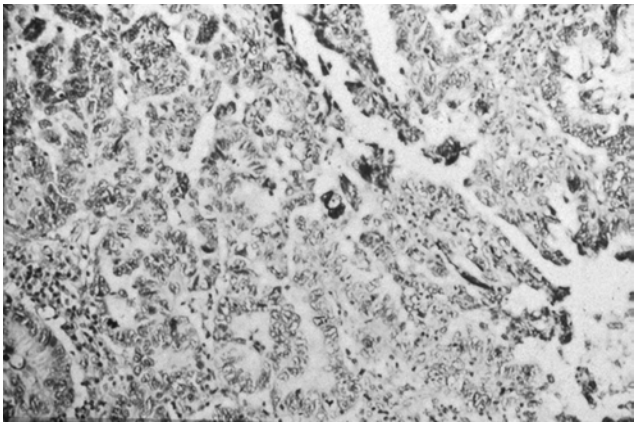

Figure 1. — Case 1: Uterine malignant mixed Müllerian tumor (HE x 100).
Figure 2. — Case 1: Uterine malignant mixed Müllerian tumor (SMA x 100).
Figure 3. — Case 2: Uterine malignant mixed Müllerian tumor (HE x 100).
Figure 4. — Case 2: Uterine malignant mixed Müllerian tumor (HE x 100).

The patient underwent chemotherapy once again, but she received only three courses of carboplatin (400 mg/m^2) due to systemic complications. She died because of recurrent disease 26 months after initial surgery for uterine MMMT.

Case 2

The patient, a 81-year-old gravida 4, para 2, postmenopausal Greek woman, presented with a complaint of abnormal uterine bleeding. She had history of breast cancer Stage IIB previously treated with modified radical mastectomy 78 months ago. Postoperatively, she received adjuvant chemotherapy and radiotherapy. She also received 20 mg TAM daily for 73 months. Her family history revealed no evidence of cancer among the first-degree relatives.

A gynecologic examination did not reveal any abnormal findings. There were no palpable inguinal lymph nodes and the rest of pelvic examination was also normal.

Preoperative CT of the abdomen and pelvis, and abdominal U/S revealed irregular endometrial thickening. Preoperative CT of the chest, chest X-ray, IVP, colonoscopy, and urethrocystoscopy were normal. Hysteroscopy and endometrial biopsy revealed uterine MMMT. Preoperative CA-125 was normal.

During exploratory laparotomy, the patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy, total omentectomy, pelvic and para-aortic lymph node dissection.

Histopathology revealed uterine MMMT. The epithelial component of uterine MMMT was adenocarcinoma and the mesenchymal component was endometrial stromal sarcoma (Figures 3 and 4). The uterine tumor did not invade the myometrium. The ovaries and omentum were normal. The peritoneal washing smear was positive for malignant cells. The final diagnosis was Stage IA uterine MMMT according to FIGO staging system 2009 [8, 9].

The patient underwent postoperative adjuvant chemotherapy. She received six courses of cisplatin (75 mg/m^2) and paclitaxel (175 mg/m^2). She also received postoperatively 5,000 cGy external and 2,000 cGy of intravaginal radiotherapy.

Follow-up at 24 months after initial surgery for uterine MMMT, with CT of the chest, abdomen, and pelvis, abdominal U/S, chest X-ray, IVP, colonoscopy, and urethrocystoscopy, revealed no evidence of recurrence.

Discussion

Uterine MMMT is a biphasic tumor of the female genital tract composed of both malignant epithelial and mesenchymal components [1, 2]. The epithelial component of

uterine MMMT may be serous, endometrioid, clear cell, squamous or undifferentiated [2, 10-12]. The mesenchymal component of uterine MMMT may be homologous or heterologous. Homologous types contain only mesenchymal elements normally found in the uterus (endometrial stromal sarcoma, fibrosarcoma, leiomyosarcoma or undifferentiated sarcoma) [10-12]. Heterologous types contain some mesenchymal elements that are not usually found in the uterus (rhabdomyosarcoma, chondrosarcoma, osteosarcoma or liposarcoma) [10-12]. In the patients studied, the epithelial component of uterine MMMT was adenocarcinoma in both cases. Also, the mesenchymal component of uterine MMMT was leiomyosarcoma in one case and endometrial stromal sarcoma in the other case.

Although the precise histogenesis of uterine MMMT is still uncertain, there are four main theories regarding it: collision, combination, conversion, and composition [2, 10, 13-14]. Most uterine MMMT are monoclonal with the epithelial component being the driving force [2, 15]. However, a small subset of uterine MMMT is true collision tumors, consisting of independent unrelated carcinomas and sarcomas [2, 16]. It may be of prognostic significance to identify this subset of uterine MMMT [2].

TAM is a non-steroidal selective estrogen receptor modulator (SERM) that has potent anti-estrogenic activity in the breast, while displaying weak estrogen activity in the endometrium [17, 18]. A significant side-effect of TAM treatment, in postmenopausal women with breast cancer, and appears to have a proliferative effect on the endometrium [19]. This side-effect is correlated with the development of various endometrial pathologies, including hyperplasia, polyps, carcinoma, and sarcoma [17-19]. There is also increased risk for the development of uterine MMMT especially in postmenopausal patients with prolonged adjuvant TAM treatment [20-22]. Other risk factors for the development of uterine MMMT are: obesity, nulliparity, exposure to exogenous estrogens, and pelvic radiation [2, 7].

There may be an association between prolonged adjuvant TAM treatment (> five years) and the development of uterine MMMT [19]. As both epithelial and stromal cells in the uterus express estrogen receptors, it is possible that prolonged weak estrogen activity of TAM treatment in the endometrium may be associated with the development of uterine MMMT [20, 23]. Both patients presented were postmenopausal and received TAM treatment for seven and 73 months respectively.

Uterine MMMT most commonly occur as a solitary, large, soft, polypoid mass with hemorrhagic and necrotic regions [10, 12]. It usually fills and distends the endometrial cavity and invades the myometrium [10, 24].

The clinical presentation of uterine MMMT is usually nonspecific. The most common presenting signs and symptoms are: abnormal uterine bleeding, abdominal/pelvic pain, and abdominal/pelvic mass [10, 12, 24]. The patients presented with a complaint of abnormal uterine bleeding.

The nonspecific nature of signs and symptoms in patients with uterine MMMT still renders preoperative diagnosis exceptional. Magnetic resonance imaging (MRI) findings are usually not pathognomonic and differential diagnosis includes endometrial cancer [25]. The final diagnosis of uterine MMMT is usually histological and sometimes is possible only after hysterectomy [12]. In many cases (> 50%), patients diagnosed at advanced stage disease [2, 12, 26-28]. In the patients presented, preoperative CT of the abdomen and pelvis, and U/S revealed irregular endometrial thickening.

Uterine MMMT primarily spread via lymphatics, similar to endometrial carcinomas [29]. Also, most patients with uterine MMMT die from local recurrence in the pelvis and abdomen rather than from metastatic disease [2, 30].

The most common sites of metastasis in uterine MMMT are: lung (49%), peritoneum (44%), pelvic and para-aortic lymph nodes (35%), adrenal gland, bones, heart, pericardium, and brain [2, 10, 12, 24].

However, recurrence rates after initial surgery and postoperative adjuvant therapy are between 47% - 64% [10, 28, 30]. Recurrence rate is 44% for homologous and 63% for heterologous uterine MMMT [30], and most of them are associated with distant metastases [10, 28].

Treatment of choice in patients with uterine MMMT is: total abdominal hysterectomy with bilateral salpingo-oophorectomy, total omentectomy, pelvic lymphadenectomy, para-aortic lymph node sampling, and maximal tumor debulking [10, 24]. However, the beneficial role of lymphadenectomy remains undetermined [31]. In node-negative patients, extended lymphadenectomy actually offers survival benefit [31, 32]. In node positive patients extended lymphadenectomy cannot improve survival due to the systematic spread of the disease [31, 32]. The patients presented underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy. Only one of them underwent pelvic and para-aortic lymph node dissection.

Uterine MMMT should be considered as metaplastic carcinoma and adjuvant treatment should be similar to that directed against aggressive subtypes of endometrial adenocarcinoma [2]. Also, high rates of postoperative local and distant relapse necessitate effective postoperative adjuvant chemotherapy and/or radiotherapy [6, 33, 34].

Adjuvant radiotherapy includes external pelvic radiotherapy and/or brachytherapy [24]. Data regarding the efficacy of adjuvant radiotherapy in patients with uterine MMMT are conflicting [24, 32, 35, 36]. It usually reduces the incidence of local recurrences; however, a beneficial effect on survival is inconsistent [35, 36]. The patients underwent postoperatively 5,000 cGy of external and 2,000 cGy of intravaginal radiotherapy.

Adjuvant chemotherapy should be similar to that directed against aggressive subtypes of endometrial adenocarcinoma [2, 6]. Combination chemotherapy with paclitaxel and carboplatin is effective in patients with uterine MMMT [37]. However, in advanced stage as well as in recurrent disease, adjuvant combination chemother-

apy with ifosfamide and paclitaxel should be considered [38]. The patients in this study underwent postoperative adjuvant chemotherapy.

Also, ErbB-targeted therapies might be a new therapeutic approach in patients with uterine MMMT and positive EGFR and erbB-2 receptors [24, 39-41].

Despite treatment modality, uterine MMMTs generally have poor prognosis. Advanced stage at initial diagnosis and intrinsic aggressiveness of uterine MMMTs, may explain dismal prognosis observed in those patients [2, 10]. Although very rare, they are associated to 16.4% of deaths caused by uterine malignancies [10, 12].

Prognostic factors for uterine MMMTs are: stage, age, histologic type of epithelial component, and presence of heterologous mesenchymal component [30, 42, 43]. However, the stage of the disease at initial diagnosis, is the most important prognostic factor [10, 42, 43].

The median survival in patients with uterine MMMT ranges between 16 and 40 months with death usually occurring within one to two years after initial diagnosis [10]. Also, five-year survival rates in patients with uterine MMMT are: 56% for Stage I, 31% for Stage II, 13% for Stage III, and 0% for Stage IV [28]. One of the patients in this study died 26 months after initial surgery due to uterine MMMT.

Conclusion

Uterine MMMT is a rare, highly-aggressive, and rapidly-progressing tumor associated with a poor prognosis. Postmenopausal patients, with prolonged adjuvant TAM treatment for breast cancer, are at increased risk for the development of uterine MMMT. All these patients should be closely monitored, especially if they have abnormal uterine bleeding, abdominal/pelvic pain, and abdominal/pelvic mass.

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Condyloma acuminata induces focal intense FDG uptake mimicking vaginal stump recurrence from uterine cervical cancer: a case report

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Summary

The 2-deoxy-2-[^{18}F] fluoro-D-glucose position emission tomography/computed tomography (FDG PET/CT) findings of condyloma acuminata in a patient with FIGO Stage IB1 cervical cancer who had previously been treated with radical hysterectomy, pelvic chemoradiotherapy, and consolidation chemotherapy is described in this article. This case highlights the importance of considering condyloma acuminata during the differential diagnosis of abnormal vaginal FDG uptake in patients who have been treated for gynecological cancer.

Key words: FDG PET/CT; Condyloma acuminata; Cervical cancer; Recurrence.

Introduction

The immune status of patients is reported to be an important factor for the progression of condyloma acuminata [1-4]. Thus, impaired immunity induced by intensive cancer treatments may allow the patient's human papilloma virus (HPV) infection to persist and favor the development of genital condyloma acuminata. The authors present a case of condyloma acuminata occurring in a cervical cancer patient that showed a focal intense FDG uptake mimicking vaginal stump recurrence.

Case Report

A 31-year-old woman with FIGO Stage IB2 squamous cell carcinoma of the uterine cervix had been treated with radical hysterectomy followed by pelvic concurrent chemoradiotherapy, plus consolidation chemotherapy. Two months after the completion of these initial treatments, she underwent 2-deoxy-2-[^{18}F] fluoro-D-glucose position emission tomography/computed tomography (FDG PET/CT) as part of a routine follow-up program. As shown, FDG PET/CT detected intense vaginal FDG uptake, which was highly-indicative of vaginal stump recurrence (Figure 1A). On physical examination, she was found to have a one cm friable, irregular, and pigmented mass, which was limited to the vaginal stump. Biopsies of the lesion revealed squamous epithelial proliferation (Figure 2A), with prominent papillomatosis and koilocytotic cells close to the surface, which were consistent with condyloma acuminate (Figure 2B). A human papilloma virus (HPV) genotyping test was positive for the HPV 6 and 11 subtypes. As none of the other clinical or radiological findings indicated recurrent cervical cancer, the authors concluded that the vaginal FDG uptake had been caused by the condyloma acuminata. She was treated with laser therapy followed by ten weeks of topical 5% imiquimod cream. On another FDG PET/CT conducted six weeks after the eradication of the condyloma acuminata, the abnormal focal uptake detected in the first examination had completely disappeared (Figure 1B). The patient is currently free of disease.

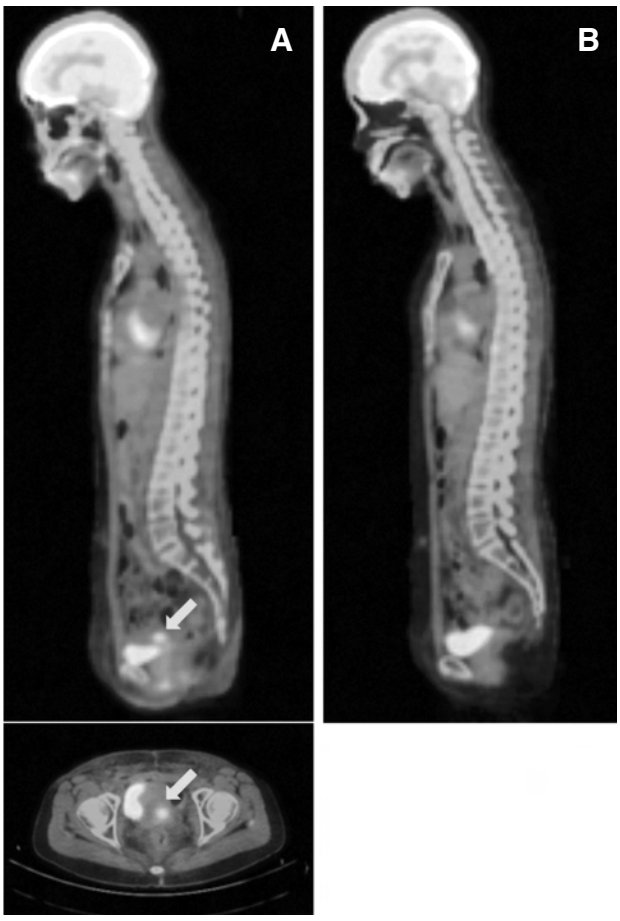

Figure 1. — FDG PET/CT fusion images obtained at the time of the diagnosis of condyloma acuminate (A), and six weeks after eradication of the condyloma acuminata (B).

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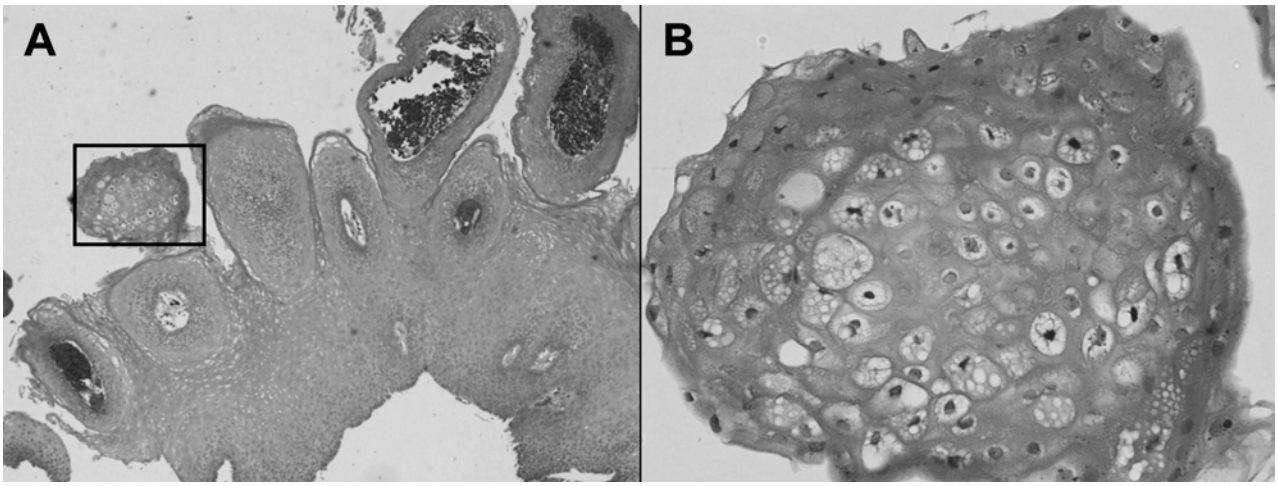

Figure 2. — Condyloma acuminata on vaginal punch biopsy (hematoxylin-eosin staining, A; original magnification x 40, B; original magnification x 200).

Discussion

Condyloma acuminata, which commonly develop as genital or perianal lesions, are induced by infection with certain types of HPV, usually HPV-6 or HPV-11 [5]. The immune status of patients is reported to be an important factor for the progression of condyloma acuminata [1-4]. According to previous reports, anal condyloma recur more frequently in patients that are immunosuppressed, including HIV-infected, transplanted patients, and those undergoing immunosuppressive chemotherapy, compared to those with a competent immune system [1, 2]. Similarly, the frequent recurrence of genital condyloma in HIV-infected women or in women receiving corticosteroid therapy has also been reported [3, 4]. In the present case, the impaired immunity induced by intensive initial treatments might have allowed the patient's HPV infection to persist and favor the development of condyloma acuminata.

Although there have been a few reported cases in which genital condyloma acuminata developed in gynecological cancer patients after treatment with pelvic radiotherapy or systemic chemotherapy [6, 7], none of them included FDG PET/CT imaging findings. Thus, to the best of the authors' knowledge, this is the first paper to report FDG PET/CT findings of condyloma acuminata. FDG PET/CT is reported to be a reliable modality for assessing uterine cervical cancer recurrence [8]; however, cases in which the false-positive accumulation of FDG in necrotic, inflammatory, or hypermetabolic lesions was mistaken for recurrent cervical cancer have also been reported [9, 10]. The present case highlights the importance of considering condyloma acuminata during the differential diagnosis of abnormal vaginal FDG uptake in gynecological cancer patients that have undergone intensive treatments that have the potential to induce immunosuppression.

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Atypical polypoid adenomyoma mixed with endometrioid carcinoma: a case report

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Summary

The following is a description of an extremely rare tumor of the uterus, malignant atypical polypoid adenomyoma (APA), admixed with well-differentiated endometroid carcinoma, in a 29-year-old patient previously treated for sterility in whom, due to the existence of a ten-millimeter sessile tumor on the uterine corpus, verified by transvaginal ultrasonography (TVUS), a hysteroscopic resection of the anomaly was performed. The patient underwent all requisite examinations and was referred to the malignant diseases panel for an examination and a decision on further treatment. As the patient wished to preserve fertility, the authors decided to continue performing regular controls at intervals of two to three months. The first subsequent control called for a TVUS examination or one using another imaging method, with a multiple endometrial biopsy with curettage of the endocervix. The results of the first examination promised that fertility could be preserved. Therapy with medroxyprogesterone acetate (MPA) in daily dosages of 200 to 500 mg was advised, which the patient intentionally did not take. A spontaneous desired pregnancy was verified following the first control.

Key words: Atypical endometrial polipoid adenoma; Hysteroscopic resection; Fertility preservation.

Introduction

Atypical polypoid adenomyoma (APA) is a disease with a low potential for malignancy, first described as such by Mazur in 1981 [1]. Within the tumor there are proliferative endometrial glands and smooth muscle cells [2]. It manifests itself as a sessile form of tumor sized from one to two cm and features increased, irregular, and profuse uterine bleeding in premenopausal patients or is present together with infertility in patients of reproductive age. It is very difficult to diagnostically differentiate between APA and atypical endometrial hyperplasia, as well as adenocarcinoma, adenofibroma, adenosarcoma, and carcinosarcoma [3]. In any case, a definite diagnosis is determined after dilatation and explorative curettage procedure of the uterine cavity or a hysteroscopic resection of the uterine corpus, with a histopathological examination of the material obtained.

A study evaluating the risk factor of a parallel development of a carcinoma and malignant transformation of APA, is required in order to establish indications for preserving fertility [4].

The locations where APA appears most frequently include: the fundus of the uterus, the lower parts of the uterus isthmus, and the cervix. The diagnostic methods, besides transvaginal ultrasonography (TVUS) are: computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography-CT (PET-CT) imaging, as well as hysteroscopy [5]. Its incidence is higher in nulliparous women who have undergone sterility treatment, aged around 40 years, although it can also appear in perimenopausal patients [6]. No clear medical consensus exists with respect to the evolution and concurrence of APA with carcinoma of the uterine corpus, as well as the form therapy should take if fertility preservation is desired. Even more uncertainty exists, when a pregnancy does occur, regarding how to follow it through until delivery, and what procedure to implement after birth – continuing intensive controls or performing a hysterectomy [3].

Case Report

During TVUS examination of a 29-year-old patient in connection with sterility, a sessile tumor of a size of about ten mm was detected on the uterine corpus. Blood-type O Rh+, biochemical, and laboratory parameter analyses showed no deviations outside normal reference values. Colposcopy findings included: ectocervix covered with stratified squamous epithelium, SCH +, PA II group. US examination of the abdomen confirmed a liver homogeneous without lesions, a cholecyst with no pathological content, a homogenous pancreas, no enlarged paraaortic lymph nodes, a homogenous and properly sized spleen, and both kidneys with hypotonia of the pharmacokinetic (PK) system. TVUS exam revealed a uterus in anteversion/flexion (AVF), hyperechogenicity in cavity, dilated by five mm in the isthmic region with hyperechogenic change in a part of the fundus up to ten mm in size. Chest X-ray showed no signs of active pathological changes or secondary deposits in parenchyma of the lungs. Hilar structure was normal and filtration coefficient (KF) sinuses were unobstructed.

Hysteroscopic resection of the anomaly in the uterine fundus was performed under general IV anaesthesia. Following the usual procedure of cervical canal dilatation, the cervical canal and uterine cavity were hysteroscopically examined. The canal and the tube openings were observed and had conventional morphological characteristics, with a sessile tumor generally white in appearance present in the fundus, positioned somewhat laterally to the right. A resection was performed and the anomaly was completely removed and sent for histopathological analysis. Hemostasis control was performed with a bipolar thermal cauteriser, and surgery was then completed. Postoperative recovery period was uneventful.

Pathologist's report included APA, with a low potential for malignancy – a polypoid structure dominated by an epithelial component, over a smooth-muscle component. Glandular epithelium showed signs of atypia and the presence of squamous morules;

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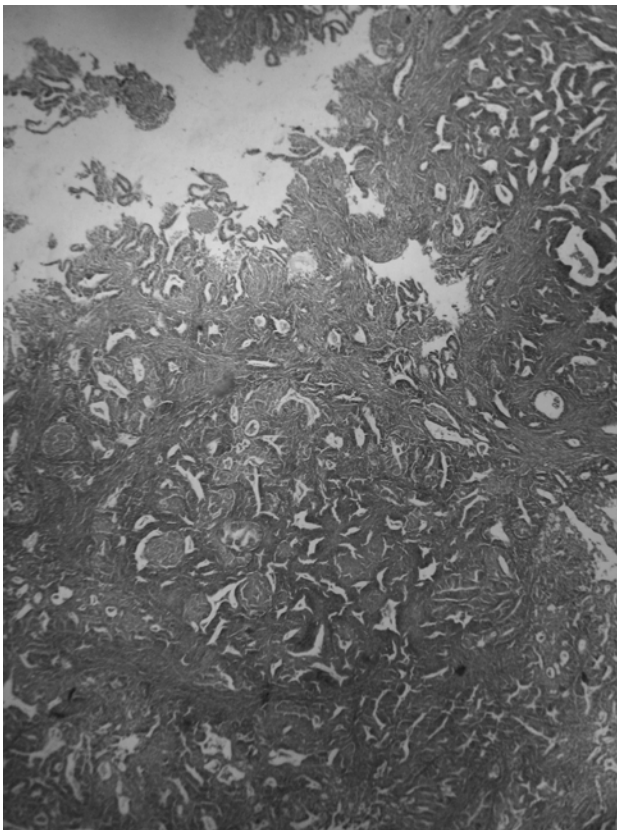

Figure 1. — Atypical polypoid adenomyoma with low-malignant potential with endometrial carcinoma.

cribriform structures were focally present as seen in well-differentiated endometrioid adenocarcinoma. The anomaly was completely removed with negative recession margins (Figure 1).

Abdominal multislice computed tomography (MSCT) and pelvis minor findings two weeks after the surgery showed a uterus dextraposed in AVF, size 93 x 80 x 67 mm, with a cavum width of up to 20 mm without focal lesions and without changes in the area surrounding the uterus. Follicles were ≤ 15 mm in size in both ovaries. No abnormalities were seen in the vagina. The abdominal findings showed no signs of spread or presence of the disease.

The patient and all documentation were presented to the malignant diseases panel. As preservation of fertility does exist among patients, the decision was taken to conduct intensive controls and regular TVUS examinations, as well as multiple biopsies of the uterine cavity. Therapy with medroxyprogesterone acetate (MPA) in daily dosages of from 200 to 500 mg was advised, until further notice.

At the next control after three months, a TVUS examination and exploratory curettage of the uterine cavity were performed. The patient had declined to apply the recommended therapy.

TVUS examination revealed a uterus in AVF, with a size of 90 x 76 x 60 mm, endometrium of 12 mm, and the adnexal findings were normal. Exploratory curettage of the uterine corpus and cervical canal performed under general IV anaesthesia and passed without complications. Pathologist's report included an APA, with a low potential for malignancy, with proliferative endometrium of standard characteristics. Endocervical mucosa were without significant changes and included squamous morules.

At the next control, a spontaneous physiological pregnancy was confirmed in the patient.

Discussion

APA is a very rare disease reported over the past 30 years and histologically characterized by proliferative endometrial glands and smooth muscle cells. The patient presented an APA, with benign changes with a low potential for malignancy; there were also histological changes representative of a well-differentiated endometroid carcinoma. It is very difficult to differentiate between APA and atypical hyperplasia, because it is unclear whether APA is a precancerous lesion for endometrioid carcinoma of the uterine corpus, or if it is a completely separate entity whose histogenesis is not sufficiently understood. Quite often it is difficult to determine the correct diagnosis, as histological forms which are detected can also be seen in malignant diseases [4]. Several cases have been reported of admixed well-differentiated endometroid carcinoma and APA, the admixture being unclear, pointing to the conclusion that a timely diagnosis and recognition of the disease must be the priority [4, 7]. There are also reports of serial changes from endometrium to carcinoma in patients without child-bearing data, which is the main reason for enhanced monitoring of patients, as the speed and progress of changes in this condition are not clear [3].

Immunohistochemical research indicates that actin, desmin, Ki67, and more recently also CD10, can provide much help in differentiating between APA and malignant diseases [8, 9]. The presence of a malignant disease and APA are based at a global level in individual cases. It is easier to set a definite diagnosis on material obtained by a hysterectomy [10]. It occurs both in women of reproductive age and older. The average patient's age is 40 years, the age range reported so far being between 25 and 73 years. The majority are women of reproductive age, and 14 out of 16 patients wish to preserve fertility [11]. The average survival time is 25.2 months; in a sample of 29 patients the range was from one to 112 months. The authors have presented a 29-year-old nulliparous patient, in whom during preparations for an artificial insemination (AI) procedure, an ultrasound examination determined the existence of a sessile tumor on the uterine corpus, without the presence of the appropriate symptomatology.

Methods for resolving this clinically-represented sessile form of tumor are hysteroscopic resection, in younger patients desiring to preserve fertility, and where symptomatology may not necessarily be present, and a more radical approach – hysterectomy, in older patients where there is also bleeding. In this patient after the requisite preparations, the anomaly was completely removed hysteroscopically and submitted for histopathological analysis. Hysteroscopic resections are proposed in four steps: the polyp is removed, the tissue surrounding the polyp is removed, as is the tissue under the polyp, and multiple biopsies are taken, in order to confirm the disease [12]. Repeated polypectomies as a part of exploratory curettages are one of the methods that can

prove a possible germinal invasion of the myometrium, although false-negative results are present in materials obtained by curettage. There are reports stating that in repeated polypectomies, in 29 cases, the disease was confirmed in 45% of all cases, 33% having a low malignant potential, and around 60% with a high potential. Some recidivation following conservative treatment has also been reported [3].

A higher APA incidence has been observed in patients subjected to estrogen therapy for longer periods, as well as in persons with Turner syndrome in whose treatment estrogen was used [13]. Hyperprolactinemia and resultant changes in hormonal status can also lead to this disease [14]. In women desiring to preserve fertility, within preparations for AI, as was the case with our patient, it is necessary to focus attention on the endometrium, together with an anamnesis of the menstrual cycle, because obvious anovulation is a factor leading to this disease [15].

APA develops in the fundus, the lower segments, and the cervix of the uterine corpus, in sizes not exceeding two cm. There are studies, based on just five presentations, where the mean value is about 3.12 cm [8].

Admixture with carcinoma and a differential diagnosis with well-differentiated endometroid carcinoma has been reported in some cases [16, 17]. Statistical analyses, based on the presentations, show that the risk of the occurrence of endometroid carcinoma in APA is 8.8% [18]. In the material obtained by a repeated curettage after resection of the polyp, the authors did not histopathologically verify the existence of endometroid carcinoma. Diagnostic methods are TVUS, where the APA is observed as a hyperechogenic sessile swelling of the interior uterine wall, and MRI, which shows a characteristic mixture of hyper- and hypoechogenicity with an irregular contrast distribution [5]. There have been reports of pregnancies in patients with this disease, but only in individual cases, and the objective is to determine the manner of control, the delivery, and postnatal treatment intended to reduce the risk of disease progression [16]. The authors have presented a patient with APA who became pregnant following resection surgery, gave birth, and for three years has been free of the disease and subject to a strict follow-up regimen [19-21]. Preserving fertility in patients of this kind certainly represents a certain risk which must be made known to them, which the authors had done with the patient presented.

Conclusion

A very rare disease whose histogenesis is not known, occurs in younger people at an average age of 40 years with sizes up to 3.12 cm. Long-term exposure to estrogen leads to the disease, and careful observation of the endometrium is required. A disease in whose treatment has no established opinions, as experiences are based on individual cases. Pregnancies and preservation of fertility are possible, while more work needs to be done in connection with the manner of delivery and postnatal control. In a period when the number of endometroid malignancies is rising, even more cases of this kind can be expected to occur.

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Perivascular epithelial cell tumor arising from polypoid adenomyoma: a case report

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Summary

The present report describes a rare case of a uterine perivascular epithelioid cell tumor (PEComa) arising from a polypoid adenomyoma. The patient, a 44-year-old woman with tuberous sclerosis, was incidentally found to have a uterine mass with malignant-appearing features on a computed tomography (CT) scan. Pathological examination of the hysterectomy specimen demonstrated that the tumor was composed of pale, spindle-shaped, epithelioid tumor cells which were positive for SMA and HMB-45. These findings were consistent with a PEComa arising from a polypoid adenomyoma.

Key words: Perivascular epithelioid cell tumor (PEComa); Uterus; Polypoid adenomyoma.

Introduction

Primary uterine perivascular epithelioid cell tumors (PEComa) are rare [1-4]. PEComas are characterized by positive melanocytic markers, such as HMB-45 [5].
The authors report a rare case of a uterine PEComa arising from a polypoid adenomyoma.

Case Report

The patient, an institutionalized, 44-year-old female with mental retardation, tuberous sclerosis, and refractory epilepsy was admitted to Shimane University Hospital in October 2011 for the evaluation and management of a uterine tumor. She had a history of ER+, PR+, HER2-, invasive ductal carcinoma diagnosed at the age of 41 that had been treated with a right mastectomy and axillary lymph node dissection in January 2008 at another hospital. She was then treated with adjuvant lupron and nolvadex from April 2008 to February 2010, after which she was treated with nolvadex monotherapy. She was then followed with semi-annual computed tomography (CT) imaging and bone scintigraphy.

CT imaging performed in June 2011 was notable for uterine and right adnexal masses. The patient underwent a dilation and curettage, the pathology of which demonstrated proliferative epithelial cells and cystic, elongated, endothelial cells with no evidence of malignancy. Magnetic resonance imaging (MRI), however, suggested endometrial sarcoma or carcinosarcoma (Figure 1); therefore she was transferred to Shimane University Hospital for diagnosis and management. She then underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy in November 2011.

Pathological findings

On gross exam, the uterus, cervix, and bilateral adnexa weighed 280 g. The uterus measured 12 x 9 cm and had diffuse adenomyosis.

Two polypoid structures, measuring four cm and 2.5 cm, were present in the cavity and were histologically diagnosed as polypoid adenomyomas. These polyps consisted of clear cells in a ligulate pattern within the wall. Immunohistochemical staining revealed that these cells were SMA, HMB45, vimentin, desmin, Melan A and PR positive, and ER negative (Figure 2 A-H). These findings were compatible with a histological diagnosis of PEComa. There were no abnormal findings in the uterine cervix or left adnexa. The right ovary had a 10 x 6 cm endometrioma.

The malignant potential of a PEComa is determined with standard criteria. Two or more of the following six characteristics classify the tumor as malignant 1) Size > 5 cm, 2) Infiltrative, 3) High nuclear grade and cellularity, 4) Mitotic rate ≥ 1/50HPF, 5) Necrosis, and 6) Vascular invasion. The present

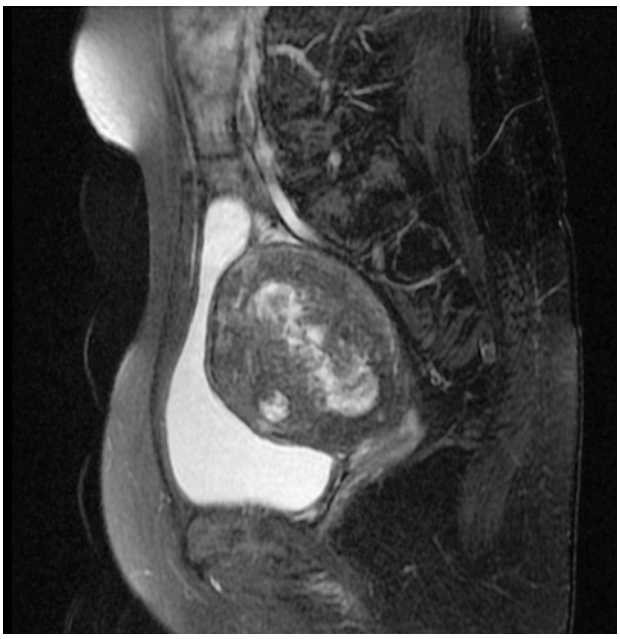

Figure 1. — MRI of preoperative axial and T2-weighted images. The submucosal tumor was identified in the uterine body. The tumor size was 7.0 x 6.4 x 4.3 cm.

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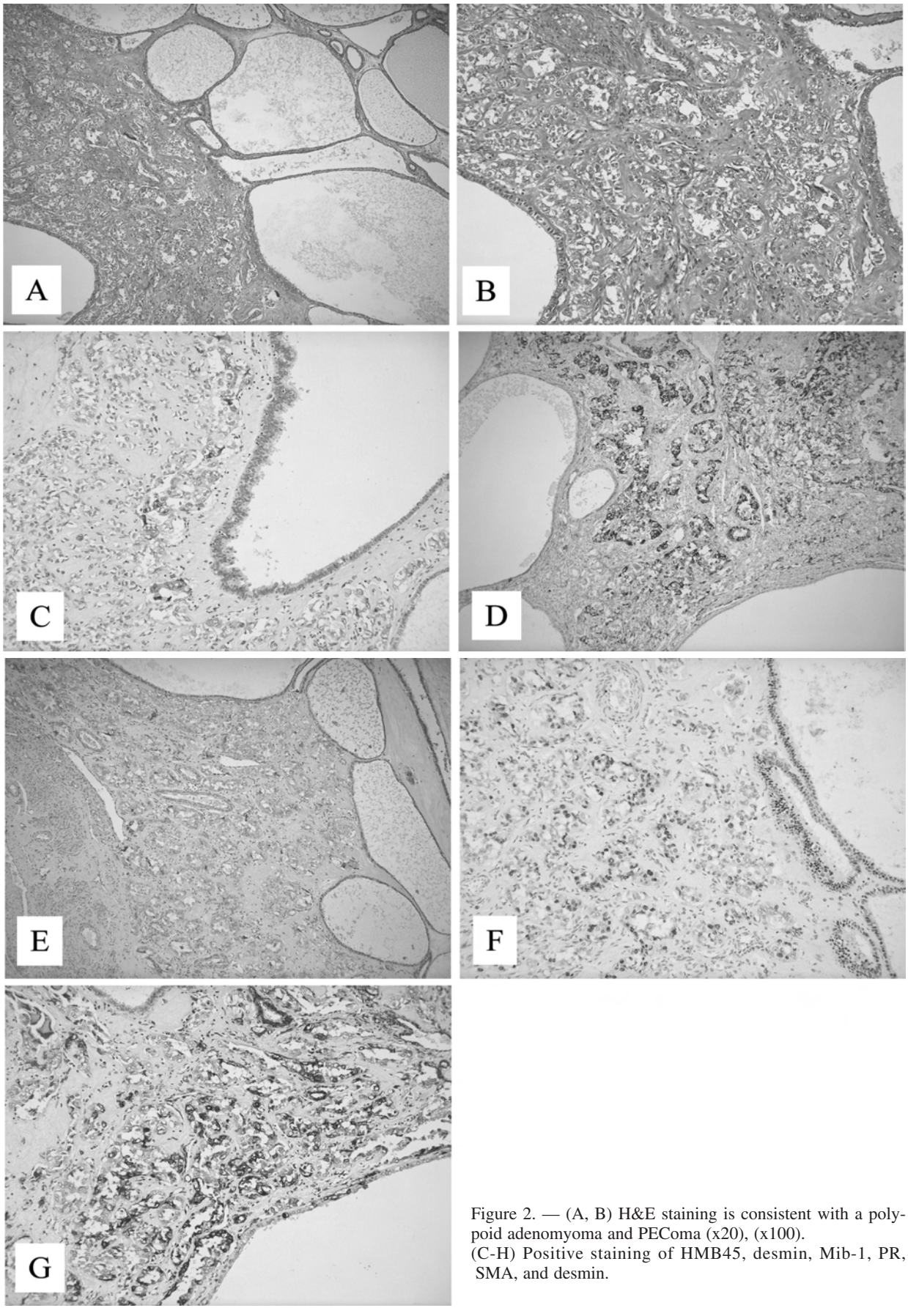

Figure 2. — (A, B) H&E staining is consistent with a polypoid adenomyoma and PEComa (x20), (x100).
(C-H) Positive staining of HMB45, desmin, Mib-1, PR, SMA, and desmin.

tumor demonstrated an infiltrative pattern, high cellularity, and nuclear grade. Vascular invasion was also suggested on hematoxylin and esoin (H&E) staining but was not confirmed by immunostaining of podoplanin. The tumor was classified as a PEComa with uncertain malignant potential.

Immunohistochemistry

Formalin-fixed and paraffin-embedded sections were dewaxed in xylene and hydrated in graded alcohol. After antigen retrieval in a sodium citrate buffer, slides were incubated overnight at 4°C with antibodies to vimentin (DAKO, Grostrup, Denmark, clone V9), SMA (DAKO, clone A4), desmin (DAKO, clone D33) ER (DAKO, clone 1D5), PR (DAKO, clone PgR636) and melanoma-associated antigen (DAKO, clone HMB-45), Melan A(DAKO, clone A103) at dilutions of 1:100, 1:100, 1:100, 1:100, 1:100, 1:50, and non-dilution, respectively. This was followed by incubation with a biotinylated linker and streptavidin-horseradish peroxidase (LSAB2 system-HRP, DAKO Cytomation, Carpinteria, CA). The signals were visualized using ABC$^+$ (DAKO Cytomation) as the substrate-chromagen at room temperature for 10 min. Sections were counterstained with hematoxylin and mounted.

Postoperative course

Given the patient's history of refractory epilepsy, the decision was made to not treat the patient with adjuvant chemotherapy. She has remained without evidence of recurrence as of the last follow-up.

Discussion

PEComa are mesenchymal tumors which stain positive for melanocyte markers such as HMB-45 [5]. PEComas include angiomyolipomas (AML), clear cell "sugar" tumors of the lung (CCST), and lymphangioleiomyomatosis (LAM). Since the pancreatic PEComa was first reported in 1996, PEComas have been identified elsewhere. Uncommon locations include the uterus, falciform ligament of liver, peritoneum, and heart [6]. Among these less common PEComas, 30% occur in the uterus, with 50 cases being reported in the literature [2-4, 7, 8]. In general, uterine PEComas present with abnormal vaginal bleeding. According to the review of 44 uterine PEComas published by Fadare *et al*., the average patient age was 45 years old (range 9-79) and 43% of presented with metastatic disease that was ultimately fatal [1].

Common sites of metastasis include the lung, liver, bone, and ovary. There appears to be a relationship with tuberous sclerosis and PEComas as 9.1% of the reviewed 44 cases (four cases), as well as the present case showed concurrent tuberous sclerosis [1]. It is possible that both arise from an alteration in tumor suppressor gene (*TSG)* function [9].

Polypoid adenomyomas account for 2% of endometrial polyps [10]. It is unclear if the presence of this entity in the present case is also related to the PEComa or tuberous sclerosis, or is coincidental. This is the first report documenting a PEComa arising in a polyp of any type. In 2008, Frorio and colleagues documented a unique case of a PEComa arising in a background of endometriosis [2]. In their report, they observed HMB-45 positive cells surrounding ectopic endometrial glands. Histopathologically, PEComas are characterized by proliferation of eosinophilic epithelioid cells or smooth muscle cell-like spindle cells. PEComas also demonstrate neovascularization around the tumor cells that is similar to what is observed in renal clear cell carcinomas. Neovascularization is not a typical feature of uterine PEComas.

Prognostic factor for PEComas have not been clearly defined. Folpe et al. reported that tumor size (larger than eight cm in diameter), frequent nuclear fission (more than 1/50 HPF), and necrosis predicted local or distant metastasis [6]. None of these features were evident in the present case. As infiltrative growth was present the authors considered the present tumor to have uncertain malignant potential.

The present case describes a rare presentation of a uterine PEComa. PEComas should be differentiated from uterine abnormalities in tuberous sclerosis patients.

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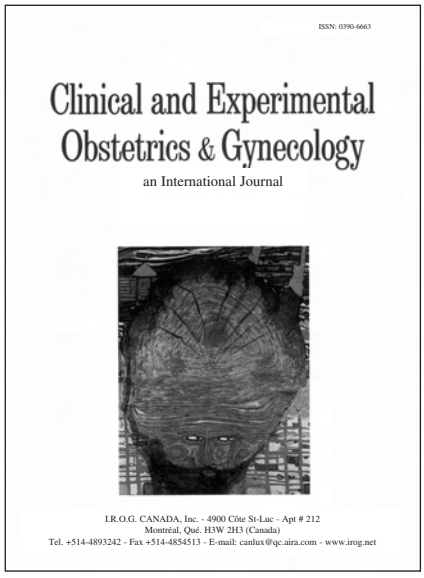

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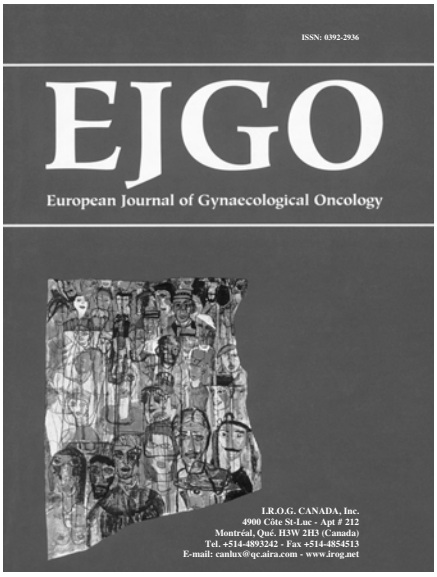

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