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Chapter I

Discovery of Estrogen Deficiency as Common Cancer Risk Factor for Highly and Moderately Estrogen Dependent Organs

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Abstract

Accumulation of non-smoker, non-drinker elderly postmenopausal female patients among smoking-associated oral cancer cases raised the plausible idea: estrogen deficiency maybe a cancer risk factor. On the other hand the extremely rare cases of young women with oral cancer regularly exhibited hormonal disorders, such as irregular menstrual cycles and infertility. Furthermore, in the history of middle-aged female oral cancer cases primary ovarian failure or complete hysterectomy were conspicuously frequent findings suggesting an estrogen deficient milieu. Also, there were many striking contradictions concerning the associations of female sexual steroids and cancer risk. Till now, breast and endometrial cancers were regarded as typically estrogen-induced tumors, particularly in post-menopausal cases. Conversely, unexplained beneficial anti-cancer effects of hormone-replacement therapy were reported against cancers at several sites even for tumors of highly hormone-responsive organs. Re-evaluation of the results of experimental and epidemiological studies, endeavoring to justify the carcinogenic capacity of estrogen, exhibited many shortcomings and controversies. Recent findings both on smoking associated and hormone related cancers added up to the same conversion; not estrogen but rather its deficiency might provoke cancer initiation. Thorough review of literary data justified that the exquisite regulatory capacity of estrogen and its surveillance on growth, development, differentiation, and metabolism are indispensable, whereas an estrogen-deficient milieu may induce a breakdown in gene-regulation. Recognition of the anticancer capacity of estrogen may provide new insights into the aetiology of malignancies and leads to new strategies for cancer prevention and cure.

Introduction

Mild or moderate estrogen deficiency is a relatively frequent, pathologic state of premenopausal women, whereas at menopause ovarian estrogen production definitely declines and serum estrogen levels show further decrease during the postmenopausal years. Conversely, pathologic, excessive estrogen production is extremely rare in women.

Distinction between cancers of moderately and highly estrogen dependent tissues is necessary, based on their different epidemiological features. Oral cancer is a typical example of moderately estrogen dependent tumors and its initiation seems to be associated with a profound estrogen loss [1]. The vast majority of malignancies of moderately estrogen dependent organs occur in the late postmenopausal years of women, when ovarian estrogen production is fairly decreased [2].

However, cancers of the highly estrogen dependent organs such as breast, usually exhibit both premenopausal and postmenopausal occurrences [3,4,5]. In premenopausal cases, marked cancer prevalence in organs with high estrogen dependence suggests that the higher hormone demand of affected organs may result in gene regulation disorders even in mildly or moderately estrogen deficient milieu [6].

In spite of the different epidemiological data of these two groups of cancers the mechanism of gene regulation disorder in the background of tumor initiation cannot act through quite opposite pathways. Many literary data justify that insulin resistance, obesity and type-2 diabetes are in similarly close association with cancer risk of both highly and moderately estrogen dependent organs [7]. Conversely, the newly revealed association between estrogen deficiency and oral cancer risk indicates a contradiction to the traditional concept of estrogen induced breast cancer. This contradiction may raise the plausible question whether increased or decreased serum estrogen levels might be the common risk factors for cancers of highly and moderately estrogen dependent organs?

Risk Factors for Cancers of Highly Hormone Related Organs

Cancers of highly estrogen dependent organs are in the forefront of tumors as they are regarded as hormone associated ones. They have multicausal origin, however, in the past decades female sexual steroids, especially estrogens were presumed to be important etiologic factors. The incidence rate of so-called hormone dependent tumors such as breast, endometrial and ovarian cancers is highest in the industrialized countries and much lower in the developing countries, with a highest to lowest ratio of 20:1 [8].

Breast tumors are important subjects of cancer research as they are the most frequent cancers in the women of the Western world. Marked geographic differences in breast cancer morbidity and mortality seem to be environmental rather than genetic in origin [3]. As higher economic development is in close correlation with higher prevalence of insulin resistance and obesity, differences in breast cancer incidence may be partially associated with the epidemiological features of metabolic diseases.

Traditional concept of the carcinogenic capacity of the female sex steroid hormones was based on the epidemiological data of breast cancer cases [9,10,11,12]. *Endogenous estrogen*was regarded as a risk for breast cancer even in case of slightly elevated circulatory level [9,13,14,15]. *Exogenous estrogens* administered alone or in combination with progestin were found also to be risk factors for breast cancer [16,17,18,19]. Results of the hormone replacement therapy (HRT) of postmenopausal women supported the relative breast cancer risk in summarized population based epidemiological studies [20]. Further literary data suggested that HRT use for more than 5-10 years means an increased relative risk for mammary malignancies [21].

Breast cancer is a tumor of fairly hormone dependent tissue, exhibiting both premenopausal and postmenopausal manifestations even without exogenous estrogen treatment [4]. Epidemiological observations can hardly be explained by the carcinogenic capacity of the elevated level of female sexual steroids, especially in postmenopausal cases. Moreover, as the majority of malignancies, breast cancer is also a multicausal tumor with strong genetic associations.

Risk factors established or suspected to be associated with breast cancer are numerous [22]. Family history and genetic disposition, lifestyle including diet, physical exercise, smoking, excessive alcohol consumption and individual hormonal and reproductive factors all markedly influence the incidence of this tumor. Recently, insulin resistant states such as obesity, elevated fasting glucose, metabolic syndrome and type-2 diabetes are also regarded as strong risk factors for breast cancer [23]. Thus hormone replacement therapy (HRT) is only one of a broad range of factors for which an inconsistent association with breast cancer of postmenopausal cases has been found.

Endometrial cancer is the most common invasive malignancy of the female genital tract with both pre- and postmenopausal variants as well [24]. Known risk factors associated with increased risk for endometrial cancer are also numerous. Among others old age, metabolic syndrome, type-2 diabetes, obesity, nulliparity, alcohol use, oral contraception and HRT use are regarded as risks for endometrial malignancies [25,26].

Hyperestrogenism is regarded as a causal factor for endometrial hyperplasia, which may be the predecessor of endometrial adenocarcinoma [27]. Many epidemiologists have accepted exogenous estrogen administration as a risk factor for human endometrial cancer [28,29,30]. Increased cancer risk was established with increasing estrogen doses and with the longer duration of treatment [31].

Literary data on the endometrial cancer risk by oral contraception show controversial results. Some authors suggested a decreased cancer risk of highly hormone dependent organs associated with oral contraception explained by a decreased cumulative number of ovulations [31,32]. Recently, insulin resistance has been in the forefront as risk for endometrial carcinoma. Drugs, used for the treatment of type-2 diabetes, are important candidates for prevention and possible treatment of endometrial carcinoma [33].

A dualistic model of endometrial carcinogenesis was proposed earlier [34], whith possible importance in the evaluation of associations of HRT with endometrial cancer. According to this concept, there are two main types of endometrial carcinomas in correlation with circulatory estrogen supply. The first type is a slowly spreading, highly differentiated form, supposedly developing in association with excessive estrogen stimulation (type I). The second type is a more aggressive variant considered to arise in a relatively estrogen-deficient milieu of elderly women (type II)! The type I form of endometrial cancer, which may be

potentially associated with HRT use, is less aggressive, with a 5-year survival rate of 70-80% [35]. The type II, poorly differentiated endometrial carcinoma affects older women and the 5-year survival rate is shorter or about 60%.

Recently, a third type of mucinous endometrial carcinoma has been associated with the use of tamoxifen, which is an estrogen antagonist drug used against estrogen receptor positive breast cancers [26,36]. An important parallelism between breast and endometrial cancers is that only the estrogen receptor positive, highly differentiated, slow growing forms have been assumed to have associations with HRT use [26,37].

Ovarian cancer represents about 30% of all malignancies of the female genital tract. The age adjusted incidence rates vary from <2/100 000 women in most of Southeast Asia and Africa to >15/100 000 cases in Northern and Eastern Europe [8]. The economically advanced countries of North America and Europe show the highest rates.

In the USA an increased risk of ovarian cancer mortality was associated with postmenopausal estrogen use based on a large prospective study [38]. A collaborative reanalysis of European studies found a relative risk of ovarian cancer related to ever use of HRT [39]. Recently, in a cohort study a strong relationship was established between duration of estrogen therapy and risk of ovarian cancer [40].

The incidence of ovarian cancer shows a steady increase with age. Reduced risk of the disease is consistently associated with high parity and oral contraceptive use [35]. Energy rich diet and insulin resistance have also been related to ovarian cancer [41].

In conclusion, the carcinogenic capacity of circulating estrogens became a prevailing concept at the turn of the millennium, particularly in association with the malignancies of highly hormone dependent organs [42].

Controversial Associations of Hormone Replacement Therapy and Cancer Risk

Hormone replacement therapy (HRT) in postmenopausal women has been fairly widespread in the economically developed countries in the past decades. These cases supply excellent possibilities to study the associations between female sexual steroid hormones and tumor incidence. Till now, the prevailing concept is that HRT is associated with an increased prevalence of gynecological cancers [12,43,44].

However, recent clinical studies on HRT use in postmenopausal women yielded unexpected and fairly controversial associations with malignancies [45,46]. Unexplained, beneficial anticancer effects of HRT use were reported against *gastric,oral, esophageal,colorectal, cervical, liver and lung cancers* [12,44,47,48,49,50,51,52] and there are also contradictory results concerning the associations of HRT and highly hormone dependent cancers[37,53,54].

Urinary bladder cancer is a typically smoking associated tumor and its risk is three to four times higher in men than in women [55]. Striking contradictions have been published concerning the associations between HRT and urinary bladder cancer. Among HRT user women an excess risk was found for bladder cancer in a case-control study [44]. Other authors could not justify any association of hormone related factors with bladder cancer incidence in women [56]. However, in a follow up study, estrogen deficiency such as

postmenopausal status and a short reproductive period caused by menopause at young age did unfavorably affect the risk for bladder cancer in women [57].

Just as urinary bladder cancer, *upper aerodigestive tract tumors* are strongly smoking associated too, and their incidence also shows a high male to female ratio. Cancer incidence at these sites exhibits controversial associations with HRT use as well. Laryngeal cancer is the neoplasm with the largest male to female sex ratio in most populations. In a case-control study on women with laryngeal cancer, menstrual and hormonal factors did not appear to have a consistent role in laryngeal carcinogenesis [58].

Conversely, prospective clinical studies justified a protective role of long term HRT (48 months) against smoking associated tumors, such as oral, laryngeal and pharyngeal cancers among current smokers, but did not affect the tumor incidence among non-smokers[48]. The authors supposed that HRT postpones rather than prevents the smoking associated cancers by a transitory maintenance of epithelial thickness and integrity in the upper aero-digestive tract, which may counteract exogenous carcinogenic agents. In a Hungarian case control study late menopause showed a negative, whereas postmenopausal state a positive association with oral cancer risk in women [1]. Considering the results of Hungarian studies on oral cancer cases systemic hormonal effects of HRT use may both prevent and postpone the induction of oral cancer. Associations of hormonal factors and upper digestive tract cancer risk exhibited beneficial anticancer effect of older age at menopause and postmenopausal estrogen therapy [59]. These findings support the beneficial effect of good hormonal equilibrium against cancer risk.

Lung cancer is also highly smoking-associated tumor and its incidence exhibits high male to female ratio. Studies on correlations between HRT and lung cancer risk in women have reported controversial associations. HRT was associated with decreased survival in women with lung cancer [60]. Nevertheless, HRT use in postmenopausal women was associated with reduced lung cancer risk, particularly after longer duration [61]. Recently, HRT was found to be associated with reduced risk for lung cancer independent of smoking status; however, this association was strongest among never smokers [50].

HRT also has anticancer capacities at other tumor sites other than the upper aero-digestive tract such as in *stomach, colon, liver and cervix* [44,49,51,52], which can hardly be explained by a local defense mechanism against tobacco products and exogenous carcinogenic agents. HRT probably exerts this advantageous effect by means of its systemic metabolic and hormonal pathways. Late menopause and longer duration of fertile life seems to correlate with a reduced risk for gastric cancer [52].

Epidemiological evidences suggest that HRT is associated with a small but substantial increase in breast cancer risk among postmenopausal women [43]. Nevertheless, some of the clinical studies failed to identify any association between increased serum hormone levels and breast cancer [62,63]. Though HRT was regarded as breast cancer risk factor from 10 studies reviewed, only 5 showed statistically significant positive association between hormone treatment and breast cancer risk whereas, the other 5 studies could not justify this correlation [20]. In a recent study, long lasting treatment with conjugated equine estrogens did not increase breast cancer incidence in postmenopausal women with prior hysterectomy [64]. Moreover, in the Women's Health Initiative randomised trials on postmenopausal women with prior hysterectomy estrogen treatment was associated with a marked reduction in breast cancer risk [54,65,66]. Authors could not explain these unexpected findings though hysterectomy means a uniformly increased risk for women included into this study.

Positive correlation between HRT and endometrial cancer has been firmly established by epidemiological studies [28,29,30,43]. An increased risk was established with increasing doses of estrogen as well as with the length of treatment [17]. Conversely, an increased risk for endometrial cancer has not been justified in association with HRT either by the Women's Health Initiative (WHI) randomised trial [53] or by the Heart and Estrogen/Progestin Replacement Study (HERS) [67]. Moreover, further studies could justify even a decreased risk for endometrial cancer in postmenopausal women with HRT use [33].

Associations between HRT and *ovarian cancer* are fairly controversial [43]. A review of 20 studies on ovarian cancer reached no satisfactory conclusion concerning HRT risk for this tumor [68]. Five of these studies described a slightly elevated ovarian cancer risk in HRT users, whereas the other 15 studies did not. Recently, further doubts concerning associations between HRT use and ovarian cancer risk have emerged [69].

All these controversial correlations between HRT use and cancer risk for both smoking associated and hormone related tumors suggest that a thorough revision of the concept concerning the carcinogenic capacity of estrogen would be necessary.

Shortcomings of Studies on the Correlations between HRT Use and Cancer Risk

The postmenopausal period in women is a physiological model for studies on the hormonal, metabolic and gene regulation changes associated with estrogen deficiency. In healthy postmenopausal women there are physiological mechanisms to adapt to the gradual loss of estrogen signals. However, this new equilibrium comprises many risks and traps for slipping out of the regular metabolic and hormonal pathways.

An abrupt decrease in estrogen hormone levels either after a natural or an artificial menopause may cause gene regulation disturbances not only in the female reproductive system but also in many other organs. Radical or subtotal hysterectomy in premenopausal women results in a sudden, definite loss of ovarian estrogen synthesis and may cause severe consequences, especially without HRT use. Spontaneous or induced abortion also causes a transitory but shocking fall in the hormone levels.

In clinical practice about 40% of women have severe menopausal complaints usually requiring medical help [70]. Menopausal complaints are not only unpleasant, bothering symptoms but suggest thorough disorders of adaptation to estrogen loss and a possibility for altered gene regulation. The hormonal alterations affect the general health of the women, as they are risks for both cardiovascular diseases and malignancies. If estrogen deficiency is regarded as a risk for cancers these patients have a greater cancer risk in their later life as compared with women who have gradually, asymptomatically lost their estrogen signals and have no need for HRT use. These considerations suggest that cancer risk in aged women, among other factors, may depend on their endogenous hormonal characteristics, their menopausal process and on HRT use in their history.

Correct evaluation of correlations between HRT use and cancer risk requires strict selection of the patients included, proper length of the observational period and awareness of the estrogen receptor positivity of tumors.

Selection of the Patients for HRT Studies

Cancers are multicausal diseases, and the carcinogenic capacity of the physiological levels of female sexual steroids is hardly, if at all justifiable. The investigation of HRT use, as a supposed cancer risk factor on pooled, unselected population of women is misleading.

Supposedly, neither the hormone treated nor the untreated groups of women involved in HRT studies are homogenous. The marked differences in menopausal processes in women and their various effects on gene regulation may partially explain the controversies concerning the associations of HRT and cancer.

Correlations between HRT use and breast cancer risk are usually examined in two groups of involved women; with and without hormone treatment, disregarding their individual hormonal and reproductive differences and other known or suspected risk factors. In these studies the majority of HRT user women may be assumed to have severe postmenopausal complaints in connection with a failure of the adaptation mechanisms. However, women without hormone treatment may have predominantly uneventful perimenopausal period due to their good adaptation. Consequently, the epidemiological studies on breast cancer risk of HRT users reflect an additive effect of the endogenous hormonal features of the HRT user population and of their hormone treatment.

A collaborative re-analysis was performed on data from 51 epidemiological studies dealing mainly with single estrogen substitution [71]. The overall relative risk for breast cancer was as low as 1.14 when HRT users were compared with never users. The increase in the risk was very small but significant because of the great number of examined cases. However, a WHI publication could find no risk for breast cancer among women who used estrogen alone, even unopposed estrogen treatment was associated with a marked reduction in breast cancer risk (HR: 0.77) [54]. The WHI authors suggested that this reduction in breast cancer risk associated to HRT requires further investigation. This valuable study seems to be a stretching force against the old frame of carcinogenicity of estrogen.

Small differences in breast cancer risk between HRT user and untreated populations of women - even advantageously decreased breast cancer risk as a result of one-armed estrogen treatment - raise the possibility of bias and improper patient selection in the majority of studies [6].

How can we explain the results of the widespread epidemiological studies, which support the slightly but consequently higher breast cancer risk in postmenopausal women with HRT use as compared with untreated cases?

Estrogen deficiency seems to be a cancer risk factor, especially for highly estrogen dependent organs. Women belonging to the group of HRT users supposedly have severe postmenopausal complaints based on failure of their adaptation. Consequently, HRT users have a higher risk for breast cancer initiation as compared with complaint-free women without treatment.

Presumably, the beneficial effect of HRT use may decrease the rate of new cancer initiation in postmenopausal women. However, the pre-existing subclinical cancers induced by the pre- or perimenopausal hormonal disorders cannot be completely destroyed. Consequently, an elevated breast cancer incidence may remain in the group of endangered women with severe endogenous hormone deficiency and/or defective adaptation mechanism in spite of HRT use. These considerations may explain the slightly but consequently elevated

breast cancer risk among HRT users as compared with untreated women with physiological adaptation.

Furthermore, the estrogen deficiency theory justifies that in a methodologically stronger WHI study an unexpected, beneficial effect of HRT use against breast cancer was observed [54]. One-armed HRT was applied on 10 793 women with prior hysterectomy by conjugated estrogen and it was found to be associated with reduction of breast cancer risk. This result justifies the importance of selection of homogenously endangered patients for epidemiological studies and supports the protective role of estrogen against breast cancer.

The effects of adaptation disorders on breast cancer risk should be prospectively studied via comparison of untreated patients with and without postmenopausal complaints. Moreover, associations of HRT use and breast cancer risk might be correctly examined if the cases and controls have similar menopausal and reproductive histories and their further risk factors are also matched.

Dynamics of Cancer Growths and the Length of Observational Period

Dynamics of breast cancer initiation and promotion is still poorly understood [72,73,74,75]. Thus, firm causal conclusions cannot be established from the associations between hormone treatment and breast cancer found on inhomogeneous female populations.

Studies on HRT user women failed to clarify whether hormone treatment apparently stimulates induction of cancers de novo by initiating mutations, or facilitates the growth of pre-existing, small tumors [37].

The tumor doubling time (TDT) concept is useful for assessment of the duration of prediagnostic stage of cancers. It is defined as the time for a tumor volume or cell number to double once [76]. TDT depends on many variables such as rate of cell division, proportion of actively dividing cells, rate of apoptoses, angiogenetic potential, intermitotic interval, shedding of tumor cells, ratio of tumor and stromal cells and so on. These numerous factors explain the wide range of estimations of TDT in breast cancer [77,78]. Current data show that a median TDT for human cancers may be 50-100 days [76].

A size of 1 cm in diameter is more or less the smallest tumor to be diagnosed clinically. This corresponds to rough and ready 10^9 cells but this number depends strongly on the tumor cell size [78]. This means that on average at least 1750-3500 days (5-10 years) are necessary from the initial mutation to a clinically and/or mammographically detectable tumor mass [79]. However, this calculation is true for a tumor only in case of ideal circumstances and does not consider the cell loss by apoptosis, death and shedding [76].Further, there are no data concerning the time demand of the transition from an intraepithelial, non-invasive cancer to an invasive one, which will further prolong the period from tumor initiation to clinical cancer diagnosis [37]. In reality, the estimated time from initiation to a diagnosable cancer might be much longer than 5-10 years.

Considering the theories on tumor growth dynamics, the presumable time between supposed initiation of breast tumors by HRT and clinical detection seems to be at least 10 years or even longer [78]. All the studies showing associations between HRT and breast cancer are based on far shorter observational periods.

Awareness of Estrogen Receptor Positivity of Cancers

Mechanisms of initiation versus promotion of hormone dependent cancers, particularly breast cancer, are only scarcely understood. A widespread theory is that hormonal influence of breast cancer is necessary from the primordial mutation to clinical cancer diagnosis after many years [67].

The pathophysiological prerequisite for the initiation and progression of hormoneinduced cancer in postmenopausal women and its association with HRT use is the presence of active estrogen receptors in the target tissues [37]. However, it is a well-known fact that estrogen receptor expression is fairly different in breast cancers and there are also receptor-negative tumors. Unfortunately, the studies, which formed the current view on the HRT-induced cancer risk, did not incorporate biochemical or immunohistochemical data to clarify levels of estrogen receptor expression of breast cancers. These shortcomings may also explain the controversial associations of HRT and breast cancer risk and the results become questionable.

Doubts concerning HRT induced cancer initiation. Majority of breast cancers detected in association with HRT use are histologically well differentiated and have a relatively good prognosis [37]. These are usually slowly growing cancers and do not metastasize early. Generally, the low-grade breast cancers with low malignancy are estrogen receptor positive. As hormone receptors are presumably the prerequisites for the theoretical impact of HRT use on breast cancer initiation, only the differentiated, receptor positive cancers with slow progression may be associated with hormonal influences.

However, initiation of both ER-positive and ER-negative breast cancers may be equally possible based on the estrogen deficiency theory. Gene regulation disturbance caused by estrogen loss does not require normal ER signals; in contrast, overwhelming growth factor activities become predominant versus non-liganded, altered ERs. In this manner estrogen deficiency may induce highly differentiated, receptor-positive or poorly differentiated receptor-negative cancers.

ER-positive breast cancers are predominantly slow growing tumors requiring longer period between presumed HRT-induced initiation and clinical cancer diagnosis as compared with undifferentiated, receptor negative ones. Consequently, for hormone receptor positive, highly differentiated cancers an even much longer observational period would be necessary to establish the correlation between HRT use and breast cancer risk.

By contrast, estrogen deficiency induced breast cancer may be initiated in the pre- or perimenopausal period by moderate estrogen loss previous to the beginning of HRT. At menopause a further, abrupt decrease of estrogen level may provoke postmenopausal complaints and at the same time, faster growth of the pre-existing cancer. However, HRT use will alleviate the complaints and diminish the aggressivity of early cancer. This explanation illuminates both the possibility of clinical cancer diagnosis as early as after 4-5 years of HRT use and the lower aggressivity of cancers associated with hormone treatment.

Doubts concerning HRT induced cancer promotion. Dietel et al. proposed that as the observation time of women with HRT use is not long enough, hormones may not initiate but may perhaps accelerate the tumor growth leading to an earlier clinical discovery [37]. A contradictory result with HRT associated promotion of breast cancer is the slower growth, reduced aggressivity of tumors and longer survival among HRT user women as compared with those who did not take HRT. Nevertheless, as only hormone dependent tumors may be affected by HRT and hormone receptor expression means a higher differentiation of breast

cancers, it is not probable that hormone treatment induces dedifferentiation and more rapid progression of tumors.

Cancer initiation and promotion are generally not contradictory processes. There are many factors, including hormonal signals, which may induce both cancer initiation and promotion. Increased rates of tumor initiation and progression have been observed in compensatory hyperinsulinemia associated with insulin resistance. Elevated insulin and concomitantly elevated IGF levels are proven risk factors for both breast cancer initiation and progression [23]. In case of another hormone, such as estrogen, an inverse effect on tumor initiation as compared with tumor promotion is hardly justifiable.

Estrogen Deficiency and Cancer Risk in Postmenopausal Women

Cancer development requires many years from initiation to clinical appearance, and this is valid also for estrogen deficient postmenopausal women, especially if the main exogenous cancer risk factors are missing. The majority of postmenopausal cancers may be initiated at the perimenopausal or postmenopausal estrogen deficient period and the clinical manifestation occurs much later. As the average age at menopause is about 50 years in Hungary, the age above 60 may be especially dangerous for clinical appearance of malignancies at moderately estrogen dependent sites in women [1].

Smoking associated tumors can be regarded as typically *moderately estrogen dependent malignancies* such as cancers of the oral cavity, pharynx, larynx, lung and urinary bladder. Their incidence exhibits very high male to female ratios and has controversial correlations with HRT use. Recent literary data support that postmenopausal hormone therapy seems to have advantageous anticancer impact on these types of cancers [6]. These observations suggest that estrogen loss in postmenopausal women may have crucial role in development of smoking-related cancers, which seems to be independent of smoking habits [1,81,82].

Oral cancer is a typically smoking-associated tumor with high male to female ratio and is regarded as a moderately hormone dependent cancer type. The almost exclusively *postmenopausal state* of female oral cancer patients suggests that estrogen deficiency has crucial role in the development of this tumor [1,81]. The length of the interval between menopause and tumor onset proved also to be decisive factor in the clinical appearance of oral cancer. A fairly long mean interval between menopause of oral cancer cases and tumor diagnosis (near 17 years) suggested an important role of estrogen deprivation in oral cancer initiation. The longer theperiod of estrogen deficiency the higher the possibility of postmenopausal tumor manifestation.

An early menopause, such as premature ovarian failure under 40 years of age or premenopausal hysterectomy with or without ovariectomy means a *shorter hormonally activereproductive period* and may have thorough consequences affecting gene regulation. A significantly higher ratio of young age (<45 yrs) at menopause among the female oral cancer cases as compared with the age-matched tumor-free controls was demonstrated [1].

Moreover, a *sudden loss of the estrogen signal* may be an especially dangerous shock for the regulatory mechanisms both in the highly and moderately estrogen dependent organs.

Hysterectomy with or without ovariectomy proved to be a high risk factor for oral cancer among women in our epidemiological study [1,81].

Cancers of the highly estrogen dependent organs such as breast cancer may also occur in older postmenopausal cases. In these women a good hormonal equilibrium may be presumed during their reproductive period, which defends the highly hormone dependent organs from cancer initiation. The first shock of estrogen loss arises in the perimenopausal period, which may serve as cancer initiator. However, the continuously decreasing ovarian estrogen synthesis during postmenopausal life may be a sword of Damocles for all organs.

Moderate Estrogen Deficiency and Cancer Risk in Premenopausal Women

Carcinogenic effects of marked estrogen deficiency may easily be studied in women, since their life is clearly separated into premenopausal and postmenopausal periods. However, the long-term systemic effects of mildly or moderately decreased estrogen levels in premenopausal women maybe hard to clarify.

In premenopausal women there are many pathological states predisposing to mild or moderate estrogen deficiency. Ovarian insufficiency may result in long or irregular menstrual cycles and unexplained infertility. Chronic anovulation and ovulatory dysfunction were found also to be associated with increased prevalence of endometrial cancer [83]. Spontaneous and induced abortions provoke sudden decrease of the highly elevated estrogen level and may be regarded as transitory periods with increased inclination to disorders of gene regulation. Literary data are controversial concerning the associations of abortion and breast cancer risk; however, late termination of pregnancy at abortion seems to be a high risk for breast cancer [84]. Immunosuppressive therapy against autoimmune diseases, cancer or rejection reaction in organ transplantation cases may also induce ovarian insufficiency and risk for development of malignancies.

Polycystic ovarian syndrome (PCOS) is a complex disorder that is presumably caused by a large number of different genetic abnormalities. PCOS is the most common endocrinopathy of women in the reproductive age [85,86,87]. PCOS seems to be a pathological model of hormonal and metabolic alterations of postmenopausal status in premenopausal women. It may usually be manifested by menstrual disorders, anovulation, infertility, hirsutism and obesity and means a conspicuously increased risk for cancers at highly estrogen dependent sites [85,86,88].

Some authors suppose that unopposed estrogen levels continuously stimulate ERs in women with PCOS, which maybe a risk for endometrial and breast cancers [86]. However, insulin resistance and hyperinsulinemia in patients with PCOS are associated with high ovarian androgen synthesis and estrogen deficiency [89].

Infertility is the most sensitive indicator of hormonal disorders in symptom-free women. PCOS cases diagnosed among adolescents suggest that the disease may develop insidiously during puberty rather than at a later time of presentation of infertility [87]. Consequently, hormonal insufficiency may be long lasting enough to promote cancer development in the young adulthood of affected women.

PCOS has a great significance as latent, undiagnosed cases are relatively numerous among the female population [90,91]. If the diagnosis is based on the morphological findings of ovarian pathology at either surgery or ultrasonography, then up to 20% of unselected women have been reported to be affected [90,92]. Or about 25% of these women have no clinical symptoms although many do have laboratory findings suggesting endocrinological and metabolic disturbances associated with PCOS such as hyperandrogenism and hyperinsulinemia [23].

PCOS-associated endocrine alterations mean risk factors for breast and gynecological cancers. In otherwise healthy, young premenopausal patients with unexpected malignancies a thorough endocrinological examination should be performed.

Conspicuous Differences between Cancer Epidemiology of Highly and Moderately Estrogen Dependent Organs

Epidemiological studies on the incidence rate of moderately estrogen dependent cancers provide the possibility to compare the data of the two genders, whereas in case of highly hormone dependent cancers of women this advantage is excluded.

The most important source of misunderstandings and misinterpretations in the epidemiology of highly estrogen dependent breast cancer may be that its exhibition is almost exclusively restricted to one gender. On the contrary, in case of the moderately hormone dependent oral cancer, gender-related epidemiological differences and their trends have raised many questions to be answered [93]. Moreover, the gender-related differences in oral cancer epidemiology served as leaders on the route from smoking associated cancers to the discovery of estrogen deficiency induced carcinogenesis.

Recently estrogen and its receptors are regarded as pivotal gene regulators affecting cell growth, proliferation, differentiation and metabolism [94]. Cells of all tissues and organs have estrogen receptors with variable expression levels and they are deeply influenced by estrogen receptor signal induced mechanisms even out of the female genital tract. Therefore, assumption of a strict distinction or even inverse mechanisms between carcinogenesis in highly and moderately estrogen dependent organs is not reasonable.

Cancers of the *highly estrogen dependent breast, endometrium and ovary* have certain special characteristics even without exogenous hormone treatment. They have no sharp distinction concerning the incidence rate between premenopausal and postmenopausal cases.

Epidemiological data on cancers of the highly estrogen dependent organs show a relatively wide range of ages among patients. Moreover, among breast cancer cases at least 25-30% of the affected women are premenopausal [3]. Recently, in a prospective study on breast cancer cases over than 50% of the patients were premenopausal [4]. These data suggest that for the highly estrogen dependent organs even a mild or moderate estrogen deficiency in young, premenopausal cases is enough to initiate gene regulation disorders. Breast cancer, which is a typical example of cancers of the highly hormone related tissues, is quite uncommon in women younger than 30 years of age. Thereafter, the breast cancer risk steadily increases throughout life and after menopause the upward slope of the curve is much less

steep [3]. Endometrial cancer may also occur in young women and up to 30% of the cases may be premenopausal [5].

These epidemiological data seem to support the carcinogenic capacity of moderate estrogen deficiency in young, premenopausal cases affecting both tumor initiation and promotion. Development of decreased or instable estrogen levels may be originated in adolescence and it may be completely symptom-free apart from fertility problems. Organs with high estrogen demand may be disturbed in their gene regulation at very young ages by hormonal disorders. Insidious, mild estrogen deficiency in puberty may result in development of diagnosable breast, endometrial or ovarian tumor in young adults.

Later, the disorders of the hormonal equilibrium show a continuously increasing prevalence with age. Transitory or definite estrogen loss in the reproductive period caused by endogenous ovarian insufficiency, abortions, therapeutic measures or other causes may result in increasing prevalence of cancers in the highly hormone dependent organs.

Among premenopausal PCOS cases with hormonal disorders an increased prevalence of cancers could be observed preferentially in the breast, endometrium and ovary [85,86,88,95]. In a mortality study on patients with PCOS breast cancer proved to be the leading cause of death [85].

During the postmenopausal years further increase of breast cancer incidence can be observed. Initiation of breast tumors diagnosed in cases within 1-5 years after menopause may not be associated with either the exact date of the last menstruation or with HRT use. Tumor induction in these cases may be originated from a long lasting pre- or perimenopausal hormonal deficiency. Moreover, the postmenopausal abrupt decrease of estrogen levels may help the faster growth of a pre-existing, subclinical cancer.

Cancers of the *moderately estrogen dependent organs* occur typically in older women [2] who are supposedly postmenopausal suggestive of a profound estrogen loss in association with initiation of these tumors. Moderately estrogen dependent cancers show striking differences in prevalence and age related distribution in the two genders. The male to female ratios of oral cancer showed a conspicuous dromedary-shape curve when studied in different age groups (Figure 1).

Shockingly young oral cancer cases less than 30 years of age are extremely rare. Among them the effect of traditional, exogenous risk factors such as excessive alcohol intake or tobacco may be excluded, and a low male to female ratio or even predominance of female cases could be observed [93].

In young women oral cancer initiation requires more profound estrogen deficiency from their adolescence as compared with the highly estrogen dependent cancers. As oral cancer has a multicausal origin, a further possibility is the coexistence of mild estrogen deficiency with other non-traditional cancer risk factors.

Similar, estrogen deficient states are probably extremely rare in young boys; theoretically it is rather androgen excess, which may provoke their gene regulation disorders. These differences in hormonal disorders may explain the equalization of the male to female ratio of oral cancer among cases less than 30 years of age.

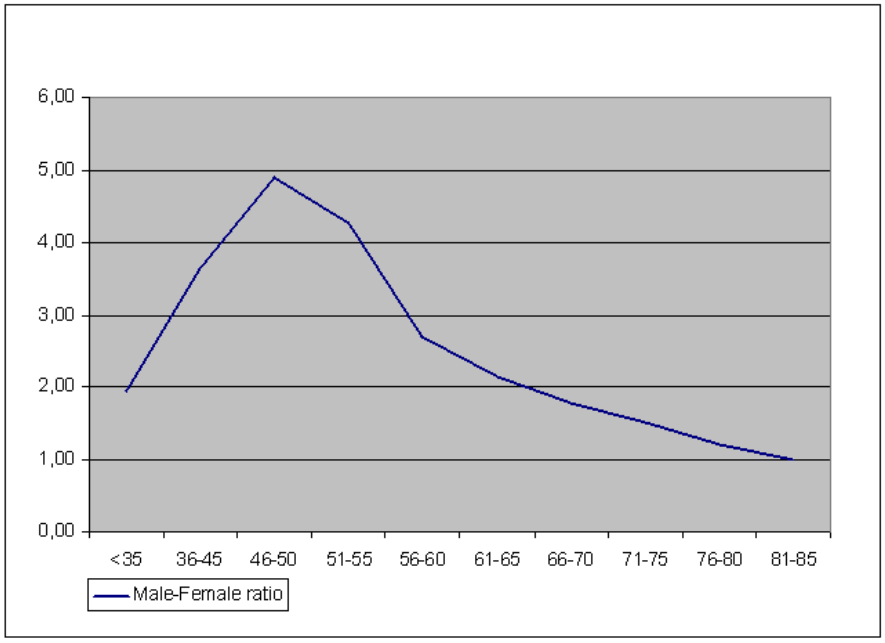

Figure 1. Male to female ratio of oral cancer patients depending on their age shows a dromedary like curve and a steep decline begins at 51-55 years of age.

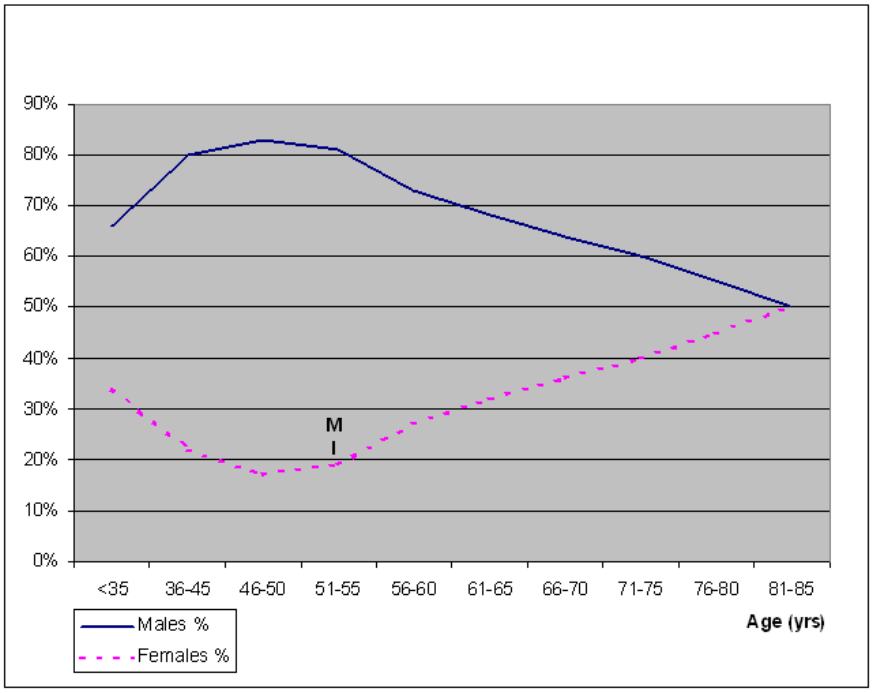

Figure 2.Percentage of male and female cases among oral cancer patients depending on their age. Between 40 and 60 years of age the male to female ratio is constantly high. At mean age of menopause (M) an increasing trend of cancer incidence among females but a decreasing trend among males can be observed.

Oral cancer incidence in adult and middle aged female cases (between 40-60 yrs) is rare as healthy women in their reproductive period enjoy the protective effect of female sexual steroids against cancers. Moreover, moderately estrogen dependent cancers are rarely associated with mild hormonal disorders. Consequently, oral cancer cases between the ages of 40 and 60 exhibit the highest male to female ratio because of the low incidence rate of women. As clinical cancer diagnosis from perimenopausal tumor initiation requires at least 10 years or more, the male to female ratio of diagnosable oral cancer remains high till the age of early 60s.

However, above the age of 60, or about 10 years after the mean age at menopause a sharply increasing ratio of female oral cancer cases as compared with males justifies the tumor inductive effect of a sudden estrogen decrease at menopause. Percentage of male and female cases among oral cancer patients depending on their age exhibits characteristic fish-shaped curves (Figure 2). These age-dependent, gender related differences in oral cancer prevalence might plausibly derive from the coarse hormonal changes between the reproductive and postmenopausal periods of women [6].

Extraovarian Estrogen Synthesis

There are recently clarified possibilities for extraovarian estrogen synthesis in postmenopausal women. In premenopausal women and estrous animals, the principal source of estradiol is ovarian, however, during reproductive senescence a significant amount of estradiol is produced extragonadally.

Female sexual steroids may be synthesized from precursor steroids by aromatase enzyme activity in many cell and tissue types included vascular endothelium, bone, brain, breast and endometrium [30,96,97,98]. Surprisingly, these are the very tissues evidently endangered by an estrogen deficient milieu and the severe consequences are cardiovascular disease, osteoporosis and ischemic injuries of the central nervous system. Similarly, aromatase activity and extragonadal estrogen synthesis in the highly estrogen dependent breast and endometrium suggest important defensive roles in postmenopausal women against cancer initiation.

Some authors regarded estrogen accumulation in the breast tissue of postmenopausal women as justification of hormone induced carcinogenesis in estrogen deficient cases [21]. However, it is not probable that a biological process serves as a facilitation of cancer development. Rather, it may be a defensive mechanism, as highly estrogen dependent tissues have increased inclination to cancer initiation in a hormone deficient milieu.

Estrogen Deficiency in Men

In men, the metabolic importance of estrogen milieu has been newly recognized. Recently, the role of sexual steroid equilibrium seems to be also essential in male physiology [99].

Complete estrogen deficiency in men is extremely rare such as aromatase deficiency and estrogen resistance [100,101]. Examinations on these sporadic male cases resulted in striking

findings concerning metabolic disorders, such as severe type-2 diabetes being resistant to usual therapy. In these totally estrogen deficient male cases type-2 diabetes may be treated by estrogen administration. Of course, these sporadic estrogen deficient male cases cannot supply any information concerning the long-term effects, even the carcinogenicity of their hormonal disorder.

Currently, the pathophysiological effects of moderate estrogen deficiency in men and its consequences are quite obscure. Based on the more conspicuous findings found in female cases, a disturbed equilibrium of sexual steroids and especially estrogen deficiency may also be important cancer risk factor in men.

Conclusion

The novel hypothesis of estrogen deficiency as a cancer risk factor may explain the controversial associations between estrogen and cancer risk. It gives an explanation as to how typically smoking associated tumors could be initiated without a smoking history and how cancers related to estrogen deficiency could be induced both in moderately and highly estrogen dependent organs. The new findings both on smoking associated and hormone related cancers might lead to the same conversion; not estrogen but rather its deficiency may provoke cancer initiation. Recognition of the anticancer capacity of estrogen may provide new insights into the aetiology of malignancies and leads to new strategies for cancer prevention and cure.

References

[1] SubaZs. Gender-related hormonal risk factors for oral cancer. *Pathol.Oncol. Res*. 13:195-202, 2007.

[2] Kumar V, Abbas AK, Fausto N, Mitchell RN: *Robbins Basic Pathology*. 8th edition Saunders Elsevier 2007.

[3] Montag A, Kumar V. The female genital system and breast Chapter 19 In: Kumar V, Abbas AK, Fausto N, Mitchell RN: Rob*bins Basic Pathology*. 8th edition Saunders Elsevier 2007.

[4] Muti P, Quattrin T, Grant BJB et al. Fasting glucose is a risk factor for breast cancer. *Cancer Epidemiol.Biomark. and Prev.* 11:1361-1368, 2002.

[5] Soliman PT, Oh JC, Schmeler KM, Sun CC, Slomovitz BM, Gershenson DM, Burke TW, Lu KH. Risk factors for young premenopausal women with endometrial cancer. *Obstet. Gynecol*. 105:575-580, 2005.

[6] SubaZs. Re-evaluation of the epidemiological associations of female sexual steroids and cancer risk. Chapter 6. In: *Estrogen versus cancer.* Nova Science Publishers, Inc. New York, 2009.

[7] SubaZs. Insulin resistance, hyperinsulinemia and cancer risk. Chapter 2. In: *Estrogen versus cancer*. Nova Science Publishers, Inc. New York, 2009.

[8] American Institute for Cancer Research /World cancer Research Fund Food, nutrition and the prevention of cancer: a global perspective. American Institute of Cancer Research, Washington, DC 252-287, 2002.

[9] Key TJ, Applyby PN, Reeves GK. Body mass index, serum sex hormones and breast cancer risk in postmenopausal women. *J. Natl.Cancer Inst.* 95:1218-1226, 2003.

[10] Berrino F, Muti P, Micheli A, Bolelli G, Krogh V, Sciajno R et al. Serum sex hormon levels after menopause and subsequent breast cancer. *J. Natl. Cancer Inst.* 88:291-296, 1996.

[11] Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE. A prospective study of estradiol and breast cancer in Japanese women. *Cancer Epidemiol.Biomark.and Prev.*9:575-579, 2000.

[12] La Vecchia C, Brinton LA, McTiernan A. Menopause, hormone replacement therapy and cancer. *Maturitas.* 39:97-115, 2001.

[13] Feigelson HS, Henderson BE: Estrogens and breast cancer. *Carcinogenesis.*17:2279-2284, 1996.

[14] Bernstein L. The epidemiology of breast cancer. *LOWAC J.* 1:7-13, 1998.

[15] Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, Banerjee S, Koenig KL, Shore RE et al. A prospective study of endogenous estrogens and breast cancer in post-menopausal women. *J. Natl. Cancer Inst.* 86:1076-1082, 1995.

[16] Colditz GA, Hankinson SE, Hunter DJ, Willett EC, Manson JE, Stampfer MJ et al. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. *N. Engl. J. Med*. 332:1589-1593, 1995.

[17] Key TJA, Pike MC. The dose-effect relationship between "unopposed" estrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk. *Br. J. Cancer.* 57:205-212, 1988.

[18] Henderson BE, Ross RK, Pike MC. Hormonal chemoprevention of cancer in women. *Science.*259:633-638, 1993.

[19] Hulka BS, Liu ET, Lininger RA. Steroid hormones and risk of breast cancer. *Cancer.*74:1111-1124, 1993.

[20] Pike M, Bernstein L, Spicer D. Exogenous hormones and breast cancer risk. In: Neiderhuber J (ed). *Current Therapy in Oncol. BC Decker*, St. Louis, MO. pp.292-302, 1993.

[21] Liehr JG. Is estradiol a genotoxic and mutagenic carcinogen? *Endocrine Rev.* 21:40-54, 2000.

[22] Brekelmans CT. Risk factors and risk reduction of breast and ovarian cancer. *Curr. Opin. Obstet. Gynecol.* 15:63-68, 2003.

[23] Bloomgarden ZT. Second World Congress on the Insulin Resistance Syndrome: Mediators, pediatric insulin resistance, the polycystic ovary syndrome, and malignancy. *Diabetes Care.* 28:1821-1830, 2005.

[24] Greenle RT, Murray T, Bolden S, Wongo PA. Cancer statistics. CA *Cancer J. Clin*. 50:7-33, 2000.

[25] Weiderpass E, Gridley G, Persson I et al. Risk of endometrial and breast cancer in patients with diabetes mellitus. *Int. J. Cancer.* 71:360-363, 1997.

[26] Horn LC, Dietel M, Einenkel J. Hormone replacement therapy (HRT) and endometrial morphology under consideration of the different molecular pathways in endometrial carcinogenesis. *Eur. J. Obst. Gyn.Repr. Biol.* 122:4-12, 2005.

[27] Cirisano FD, Robboy SJ, Dodge RK, Bentley RC, Krigman HR, Synan IS, Soper JT, Clarke Pearson DL. The outcome of state I-II clinically and surgically staged papillary serous and clear cell endometrial cancers when compared with endometrial carcinoma. *Gynecol. Oncol.* 77:55-65, 2000.

[28] Siiteri PK, Nisker JA, Hammond GL. Hormonal basis of risk factors for breast and endometrial cancer. In: Iacobelli S, King RJB, Lindner HR, Lippman ME (eds). *Hormones and Cancer*. Raven Press, New York, pp 499-505, 1980.

[29] Greenwald P, Caputo TA, Wolfgang PE. Endometrial cancer after menopausal use of estrogens. *Obstet. Gynecol.* 50:239-243, 1977.

[30] MacMahon B: Risk factors for endometrial cancer. *Gynecol.Oncol.* 2:122-129, 1974.

[31] Key TJ. Hormones and cancer in humans. *Mutat. Res.* 333:59-67, 1995.

[32] La Vecchia C, Franceschi S. Oral contraceptives and ovarian cancer. *Eur.J. Cancer Prev*. 8:297-304, 1999.

[33] Ito K, Utsunomiya H, Yaegashi N, Sasano H. Biological roles of estrogen and progesterone in human endometrial carcinoma – new developments in potential endocrine therapy for endometrial cancer. *Endocrine Journal* 54:667-679, 2007.

[34] Lax SF, Kendall B, Tashiro H, Slebos RJC, Hedrick L. Analysis of p53 and K-ras mutations and microsatellite instability suggests distinct molecular genetic pathways in the pathogenesis of uterine endometrioid and serous carcinoma. *Cancer.* 88:814-824, 2000.

[35] WHO Classification on Pathology and Genetics. In: Tavassoli FA, DevileP(eds): *Tumours of the Breast and Female Genital Organs*. WHO Publications, Geneva, Switzerland. 2003.

[36] Varras M, Polyzos D, Akviris C. Effects of tamoxifen on the human female genital tract: Review of the literature. *Eur. J. Gynaecol. Oncol*. 24:258-268, 2003.

[37] Dietel M, Lewis MA, Shapiro S. Hormone replacement therapy: pathobiological aspects of hormone-sensitive cancers in women relevant to epidemiological studies on HRT: a mini-review. *Human. Reprod.* 20:2052-2060, 2005.

[38] Rodriguez C, Patel AV, Calle EE, Jakob EJ. Estrogen replacement therapy and ovarian cancer mortality in a large prospective study of US women. *J. Am. Med. Assoc.* 285:1460-1465, 2001.

[39] Negri E, Tzonou A, Beral V, Trichopoulos D, Parazzini F, Franceschi S, Booth M, LaVecchia C. Hormonal therapy for menopause and ovarian cancer in a collaborative re-analysis of European studies. *Int. J. Cancer* 80:848-851, 1999.

[40] Lacey JV, Mink PJ, Lubin JH, Sherman ME, Troisi R, Hartge P, Schatzkin A, Schairer C. Menopausal hormone replacement therapy and risk of ovarian cancer. *J. Am. Med. Assoc.*288:334-341, 2002.

[41] Bosetti C, Negri E, Franceschi S, Pelucchi C, Talamini R, Montella M, Conti E, La Vecchia C. Diet and ovarian cancer risk: a case-control study in Italy. *Int. J. Cancer.* 93:911-915, 2001.

[42] International Agency for Research on Cancer: Monographs on the evaluation of carcinogenic risks to humans: hormonal contraception and postmenopausal hormone therapy. IARC, Lyon, France, vol 72, 1999.

[43] Diamanti-Kandarakis E. Hormone replacement therapy and risk of malignancy. *Curr. Opinion Obstet. Gynecol.* 16:73-78, 2004.

[44] Fernandez E, Gallus S, Bosetti C, Franceschi S, Negri E, La Vecchia C. Hormone replacement therapy and cancer risk: a systematic analysis from a network of case-control studies. *Int. J. Cancer.* 105:408-412, 2003.

[45] Ettinger B, Friedman GD, Bush T, Quesenberry CP Jr. Reduced mortality associated with long-term postmenopausal estrogen therapy. *Obstet. Gynecol.* 87:6-12, 1996.

[46] Grodstein F, Clarkson T, Manson J. Understanding the divergent data on postmenopausal hormone therapy. *N. Engl. J. Med*. 348:645-650, 2003.

[47] Gallus S, Bosetti C, Franceschi S, Levi F, Simonato L, Negri E, La Vecchia C. Oesophageal cancer in women: tobacco, alcohol, nutritional and hormonal factors. *Br. J. Cancer.* 85:341-345, 2001.

[48] Olsson H, BladströmAM, Ingvar C. Are smoking-associated cancers prevented or postponed in women using hormone replacement therapy? *Obstet. Gynecol*. 102:565-570, 2003.

[49] Parazzini F, La Vecchia C, Negri E, Franceschi S, Bolis G. Case-control study of estrogen replacement therapy and risk of cervical cancer. *British Med. J.* 315:85-88, 1997.

[50] Rodriguez C, Feigelson HS, Deka A, Patel AV, Jacobs EJ, Thun MJ, Calle EE. Postmenopausal hormone therapy and lung cancer risk in the Cancer Prevention Study II Nutrition Cohort. *Cancer Epidemiol.Biomark. and Prev*. 17:655-660, 2008

[51] Camargo MC, Goto Y, Zabaleta J, Morgan D, Correa P, Rabkin CS. Sex hormones, hormonal interventions and gastric cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers* Prev Oct 25 [Epub ahead of print] 2011

[52] La Vecchia C, D'Avanzo B, Franceschi S, Negri E, Parazzini F, Decarli A. Menstrual and reproductive factors and gastric-cancer risk in women. *Int. J. Cancer*. 59:761-764, 1994.

[53] Anderson GL, Judd HL, Kaunitz AM et al. Womens's Health Intitative Investigator Effects of estrogen plus progestin on gynaecologic cancers and associated diagnostic producers: The Women's Health Initiative randomised trial. *JAMA.* 290:1739-1748, 2003.

[54] Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D et al. Women's Health Intitative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterctomy: the Women's Health Intitative randomized controlled trial. *JAMA.*291:1701-1712, 2004.

[55] Hartge P, Harvey EB, Linehan WJ, Silverman DT, Sulivan JW, Hoover RN, Fraumeni JF. Unexplained excess risk of bladder cancer in men. *J.Natl. Cancer Inst.* 82:1636-1640, 1990.

[56] Cantwell MM, Lacey JV Jr, Schairer C, Schatzkin A, Michaud DS. Reproductive factors, exogenous hormone use and bladder cancer risk in a prospective study. *Internat. J. Cancer.* 119:2398-2401, 2006.

[57] McGrath M, Michaud DS, De Vivo I. Hormonal and reproductive factors and the risk of bladder cancer in women. *Am. J. Epidemiol.* 163:236-244,2006.

[58] Gallus S, Bosetti C, Franceschi S, Levi F, Negri E, La Vecchia C. Laryngeal cancer in women. Tobacco, alcohol nutritional and hormonal factors. *Cancer Epidemiol. Biomark. Prev*. 12:512-517, 2003.

[59] Freedman ND, Lacey JV, Hollenbeck AR, Leitzmann MF, Schatzkin A, AbnetChC. The association of menstrual and reproductive factors with upper gastrointestinal tract cancers in the NIH-AARP Cohort. *Cancer.*116:1572-1581, 2010.

[60] Ganti AK, Sahmoun AE, Panwalkar AW, Tendulkar KK, Potti A. Hormone replacement therapy is associated with decreased survival in women with lung cancer. *J. Clin. Oncol.* 24:59-63, 2006.

[61] Kreuzer M, Gerken M, Heinrich J, Kreienbrock L, Wichmann HE. Hormonal factors and risk of lung cancer among women? *Int. J.Epidemiol.*32:263-271, 2003.

[62] Garland CF, Friedlander NJ, Barrett-Conner E, Khaw KT. Sex hormones and postmenopausal breast cancer: a prospective study in an adult community. *Am. J. Epidemiol.* 135:1220-1230, 1992.

[63] Wysowski KK, Comstock GW, Helsing KJ, Lau HL. Sex hormone levels in serum in relation to the development of breast cancer. *Am. J.Epidemiol.* 125:791-799, 1987.

[64] Stefanick ML, Anderson GL, Margolis KL, Hendrix SJ et al. Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. *JAMA.*295:1647-1657, 2006.

[65] LaCroix AZ, Chlebowski RT, Manson JE, Aragaki AK, Johnson KC, Martin L et al. Health outcomes after stopping conjugated equine estrogens among postmenopausal women with prior hysterectomy. A randomized controlled trial. *JAMA.* 305:1305-1314, 2011.

[66] Anderson GL, Chlebowski RT, Aragaki AK, Kuller LH, Manson JE, Gass M, Bluhm E, Connelly S, Hubbell FA, Lane D, Martin L, Ockene J, Rohan T, Schenken R, Wactawski-Wende J. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. *Lancet Oncol*. 2012 Mar 6. [Epub ahead of print].

[67] Hulley S, Grady D, Busch T et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women: heart and Estrogen/Progestin Replacement Study (Hers) Research Group. *JAMA.* 380:605-613, 1998.

[68] Risch HA. Hormone replacement therapy and the risk of ovarian cancer. *Gynecol.Oncol.* 86:115-117, 2002.

[69] Shapiro S. False alarm: postmenopausal hormone therapy and ovarian cancer. *Climacteric.* 10:466-470, 2007.

[70] Grady D. Postmenopausal hormones – therapy for symptoms only. *NewEngl. J. Med*. 348:1-3, 2003.

[71] Collaborative Group on Hormonal Factors in Breast Cancer. Collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer. *Lancet.* 350:1047-1059, 1997.

[72] Adami HO, Signorello LB, and Trichopolos D. Towards an understanding of breast cancer etiology. *Semin. Cancer Biol.* 8:255-262, 1998.

[73] Hofseth LJ, RaafatAM, Osuch JR, Pathak DR, Slomski CA, Haslam SZ. Hormone replacement therapy with estrogen or estrogen plus medroxy-progesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast. *J. Clin. Endocrinol. Metab.* 84:4559-4565, 1999.

[74] Li CI, Weiss NS, Stanford JL, Daling JR. Hormone replacement therapy in relation to risk of lobular and ductal breast carcinoma in middle-aged women. *Cancer*.88:2570-2577, 2000.

[75] Wisemann RA. Breast cancer hypothesis: a single cause for the majority of cases. *J. Epidemiol. Community Health.* 54:851-858, 2000.

[76] Spratt JS, Meyer JS, Spratt JA. Rates of growth of human solid neoplasms: part I. *J. Surg. Oncol.* 60:137-146, 1995.

[77] Shackney SE, McCormack GW, JrCuchural GJ. Growth rate patterns of solid tumors and their relation to responsiveness to therapy: an analytical review. *Ann Intern Med*. 89:107-121, 1978.

[78] Spratt JS, Spratt JA. What is breast cancer doing before we can detect it? *J. Surg. Oncol.* 1985;30:156-160.

[79] Haskell CM. Thorax. Unknown primary-breast cancer in cancer treatment, 2nd ed. WB Saunders Company, 1985.

[80] Anderson TJ, Battersby S, King RJB et al. Oral contraceptive use influences resting breast proliferation. *Hum. Pathol.* 20:1139-1144, 1989.

[81] SubaZs. Estrogen deficiency as cancer risk factor. Chapter 5. In: *Estrogen versus cancer*. Nova Science Publishers, Inc. New York, 2009.

[82] SubaZs. Systemic risk factors for oral cancer: Estrogen deficiency and insulin resistance. Chapter 8. pp: 157-172 In: *Progress in Oral Cancer Research.* Ed: Frederik L. Nielsen, Nova Science Publishers, New York, 2008.

[83] Coulam CB, Annegers JF, Kranz JS. Chronic anovulation syndrome and associated neoplasia. *Obstet. Gynecol.* 61:403-407, 1983.

[84] Hartge P. Abortion, breast cancer and epidemiology. *N. Engl. J. Med.* 336:127-128, 1997.

[85] Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS. Mortality of women with polycystic ovary syndrome at long-term follow-up. *J.Clin. Epidemiol*. 51:581-586,1998.

[86] Gadducci A, Gargini A, Palla E, Fanucchi A, Genazzani AR. Polycystic ovary syndrome and gynecological cancers: is there a link? *Gynecol.Endocrinol*. 20:200-208, 2005.

[87] Sartor BM, Dickey RP. Polycystic ovarian syndrome and the metabolic syndrome. *Am. J. Med*. 330:336-342, 2005.

[88] Spritzer PM, Morsch DM, Wiltgen D. Polycystic ovary syndrome associated neoplasms. *Arq. Bras. Endocrinol. Metabol.* 49:805-810, 2006.

[89] Nestler JE, Strauss JF. Insulin as an effector of human ovarian and adrenal steroid metabolism. *Endocrin. Metab. Clin. North. Amer*. 20:807-823, 1991.

[90] Polson DW, Wadsworth J, Adams J et al. Polycystic ovaries: a common finding in normal women. *Lancet.* 1:870-872, 1988.

[91] Carmina E, Lobo RA. Polycystic ovaries in hirsute women with normal menses. *Am. J. Med*. 111:602-606, 2001.

[92] Farquhar CM, Birdsall M, Manning P et al. The prevalence of polycystic ovaries on ultrasound scanning in a population of randomly selected women. *Aust. NZ. Obstet.Gynaecol.* 34:67-72, 1994.

[93] Llewellyn CD, Johnson NW, Warnakulasuriya KAAS. Risk factors for squamous cell carcinoma of the oral cavity in young people-a comprehensive literature review. *Oral Oncol.* 37:401-418, 2001.

[94] Björnström L, Sjöberg M. Mechanisms of estrogen receptor signaling: Convergence of genomic and nongenomic actions on target genes. *Molecular Endocrinol.* 19:833-842, 2005.

[95] Pillay OC, Te Fong LF, Crow JC, Benjamin E, Mould T, Atiomo W, Menon PA, Leonard AJ, Hardiman P. The association between polycystic ovaries and endometrial cancer. *Human Reprod.* 21:924-929, 2006.

[96] Miller WR, O'Neill J. The importance of local synthesis of estrogen within the breast. *Steroids.* 50:537-548, 1987.

[97] Santen RJ, Martel J, Hoagland M, Naftolin F, Roa L, Harada N, Hafer L, Zaino R, Santner SJ. Stromal spindle cells contain aromatase in human breast-tumors. *J. Clin. Endocrin.Metab.* 79:627-632, 1994.

[98] McCullough LD, Blizzard K, Simpson ER, Öz OK, Hurn PD. Aromatase cytochrome P450 and extragonadal estrogen play a role in ischemic neuroprotection. *J. Neurosci.* 23:8701-8705, 2003.

[99] Faustini-Fustini M, Rochira V, Carani C. Oestrogen deficiency in men: where are we today? *Eur. J. Endocrinol.* 140:111-129, 1999.

[100] Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J et al. Effect of testosterone and estradiol a in man with aromatase deficiency. *New Engl. J. Med*. 337:91-95, 1997.

[101] Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B et al. Estrogen resistance caused by a mutation in the estrogen receptor gene in a man. *New Engl. J. Med*. 331:1056-1061, 1994.