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A Review Study on the Effects of Estrogen Level on Vaginal *Candida* spp. of Women with Estrogen-Related Receptor Breast Cancer

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Abstract: The aim of the present study have been reported to review the estrogen level in the patients with the breast cancer and healthy individuals. Breast cancer is one of the most common diseases in women worldwide that is characterized by uncontrolled growth of malignant cells in the mammary epithelial tissue. The estrogen was found at normal level in most patients with ER-positive breast cancer and in healthy individuals, while its high level was higher among patients with ER-negative breast cancer. Many studies show evidences about the role of estrogen at a high level on the development of breast cancer. The association between the estrogen levels and the presence of *Candida* spp. in vagina of patients with breast cancer was reviewed.

Key words: Estrogen, vaginal *Candida* spp., estrogen-related receptor, breast cancer.

1. Introduction

Candida spp. is very common type of fungi living as a normal flora in different parts of the human body [1]. Vagina is one of these parts with a variable species of *Candida* occurring as a member of the community of vaginal flora [2]. Most common species of *Candida* in vagina is *C. albicans* which represented 70% of all *Candida* species [3]. The presence of *Candida* spp. in vagina usually occurring under effects of various conditions may increase or decrease its counting and diversity and may encourage *Candida* spp. to become pathogenic fungi causing vaginal candidiasis [4].

Estrogen is a sexual hormone usually found in three forms with a high level in female and low in male [5]. Its activities in the human tissues are mainly performed through the presence of specific receptors called ER (estrogen receptors) [6]. Estrogen is found in correlation with breast cancer when its level is higher, especially in postmenopausal women [7]. There are two types of ER in patients with breast cancer which make specialist divided breast cancer into two groups starting from 1970: ER-positive and ER-negative [8]. ER-positive mostly represented a high rate of ER among patients with breast cancer compared with those with ER-negative [9].

Estrogen hormone in circulating system can have an effect on vaginal *Candida* spp. through either its effects on the immunity state of vagina [10] or direct effects on *Candida* spp. itself [11]. Vagina can be affected by estrogen through its content of ER [12]. However, sensitivity of vagina into estrogen mainly depends on the menstrual cycle in women when the level decreases in postmenopausal periods and increases in premenopausal periods [13]. Many studies try to demonstrate the relationship between estrogen level and vaginal content of *Candida* species. Some of these studies found that the effect of estrogen on growth of *Candida* spp. in vagina is concentration-dependent manner [14].

2. *Candida* spp.

Candida spp. is one of the most common types of fungi found as a normal flora in different parts of the

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human body [1]. As a genus, *Candida* contains more than 200 species that all belong to the kingdom: fungi, Phylum: Ascomycota, Subphylum: Ascomycotina, Class: Ascomycetes, Order: Saccharomycetales, Family: Saccharomycetaceae [15]. Several species of *Candida* can be found in a commensalism relationship on various surfaces of the human body such as skin, vagina and other mucosal surfaces [16] while other species can be work as saprophytic fungi with inability to live at 37 °C [15]. However, the pathogenic species which are more than 17 species cannot live in the environment outside the human body [1].

Candida spp. as one of diploid eukaryotic can take a different shape ranging from cocci, cylindrical, ovoid to elongate shape with an ability to change its shape, as in other dimorphic fungi, from yeast to pseudohyphae or to true hyphae depending on the environment condition such as pH or temperature, or different compounds such as N-acetylglucosamine or proline [15]. *Candida* spp. usually lack sexual stage and some species are reclassified in different names such as change of *Torulopsis glabrata* into *Candida glabrata* [17]. The cell wall of *Candida* spp. mainly composes of polysaccharides structure such as mannans, glucans and small amount of chitin [18].

2.1 Candida spp. in Vagina

Candida spp. can normally locate on the vaginal surface layers as one of vaginal mycobiota [2]. From 196 fungal OTUs (operational taxonomic units) of vaginal, 16 OTUs are related to the *Candida* spp. [19]. Classes of Ascomycetes and Basidiomycetes and genera of *Candida* and *Saccharomyces* are the most predominant fungi in vagina [20]. Oomycetes fungal class could also be added to previous classes as found in women with recurrent vaginal candidiasis or with allergic rhinitis [21].

Growing of *Candida* spp. on vaginal surface is usually controlled by the activity of other microorganism, especially *Lactobacillus* spp. which always is competing with fungi to adhere with epithelial layers and its preventing of yeast over growing through producing of organic acids (lactic acid) which lower vaginal pH [22]. Thus, increasing colonization of *Candida* spp. on vaginal surface under specific conditions will turn yeast into pathogenic organism causing vaginal candidiasis [3]. Vaginal candidiasis could be the most prevalence type of candidiasis in women and may show high rate of recurrent infection [23]. From the result of cohort study including 1,248 asymptomatic young women for one year, about 70% have colonization with *Candida* spp. after 1-2 visits (each visit after 4 months) and 4% after 4 visits, while 30% were never colonized by yeast during the study time [24].

Most colonization of vaginal *Candida* spp. has shown no or low symptoms and can be stimulated by many factors such as sexual activities, diabetes, using birth control or contraceptives, smoking, alcoholic, and drug addict [24]. Women at middle age are also under the risk of over growing *Candida* spp. [25]. On the other hand, growing of *Candida* spp. has been found not affected by many association factors such as bacterial vaginosis, local immunomodulators, and antifungal usage [25]. However, many symptoms can be recognized in women with vaginal yeast colonization, including pruritus, discharge increasing, dysuria, malodor, and burning with focus on two of them: vulvovaginal burning and pruritus [26]. For diagnosis of vaginal *Candida* colonization, clinical signs and symptoms are usually not enough and need to be confirmed by laboratory tests [27].

3. Breast Cancer

Breast cancer is one of the most common types of cancer all over the world. It usually develops from transforming of breast cells into malignant form under the effects of genetic and environmental factors [28]. A mutation in genetic contents of some breast cells is the most possible causative of breast cancer than environmental factors [29]. Although females are

more susceptible to breast cancer, development of this cancer in males is also highly recorded [30].

New cases of breast cancer are dramatically increased as registered worldwide. It is also considerd a second cancer type causing death after lung cancer (NCI, 2020). In the USA, more than 250,000 new cases were only registered in 2017 [31]. This number will be expected to be 276,480 new cases of women in 2020 and about 2,620 cases in men (ACS 2020). Metastatic stage of breast cancer in 2017 was also diagnosed in 154,794 women in United States [32]. However, studies provided that more than 90% of breast cancer is not at metastatic stage at time of diagnosis [33].

In Eastern Mediterranean region, breast cancer is mostly ranked first in incidence among females and it is first from top five cancers recorded in Iraq based on estimation of WHO in 2009 (WHO, 2009). However, breast cancer in Iraq has been recorded with a high prevalence rate during a time. The prevalence of breast cancer in Karbala is always registered in a high rate, especially among females [34]. This rate could be fluctuated in this Iraqi city among other types of cancers from year to year as it was estimated based on histological examination in 2009 [35]. However, young women are more suffering from breast cancer in Karbala based on stimulated study from 2009 to 2017 [36].

Breast cancer is usually diagnosed based on the histopathological characterization [37]. Molecular methods are widely used nowadays for diagnosis of breast cancer. This is because most of breast cancer can develop under the effect of alteration in molecular content by mutant during the lifetime of an individual [38]. Wakes and Winer [31] divided breast cancer based on molecular markers into three categories depending on the presence or absence either of estrogen and progesterone receptors, human epidermal growth factor and triple-negative (when absence of all three molecular markers). The three categories include: hormone receptor positive/ERBB2 negative (70% of patients); ERBB2 positive (15%-20%); and 3-triple-negative (15%).

4. Estrogen

Estrogen is one of the human body sex hormone that can be found in high level in female and in low level in male. It is mainly secreted by ovary and placenta in female and under direct effect of Follicle-stimulating Hormone (FSH) and low amount of Luteinizing Hormone (LH) hormones from the adrenal cortex in men [5]. The main functions of estrogen in female include regulation of reproduction function and secondary sex characteristics as with breast development [39]. It also plays a role in reproductive biology and sexual behavior of males [40].

There are three main types of estrogen that are naturally produced in the human body, including estrone (E1), 17β-estradiol (E2), and estriol (E3) [41]. 17β-estradiol is the most active type of estrogen with several effective roles in many physiological functions ranging from regulation of reproductive organs activities to regulation of the work of other organs such as cardiovascular, immune, musculoskeletal and central nervous system [42].

4.1 ER

Estrogen can perform its activities through attachment with specific receptors called ER which are related to the large superfamily of nuclear receptors working as ligand-activated transcription factors [7]. The ER is the main binding part with estrogen after its passive diffusion through cell and nuclear membrane. Complex producing from estrogen ligand and receptors binding will trigger specific sequences in genes' response to estrogen which is called estrogen-response elements to work in regulation of cell growth and differentiation [9]. The ER has mostly similar protein structural characteristics which make it responsible for the same functions [43]. Two conserved regions can BE

recognized in ER, one called domain C located in the middle of protein, which is binding with DNA, and one called dormain E/F in the carboxy-terminal region, which is binding with hormones. There are two types of ER: ERα and ERβ, which are encoded by two distinct genes with expression in same or different tissues [44]. ERα is the main type with variable regulation of reproductive and physiological activity in the human body [45], while ERβ plays as modulator to the biological activities of ERα. Both of ERα and ERβ have 96% identified amino acids in DNA-binding domains, while they have only 53% identified amino acids in their ligand-binding domain [46].

4.2 Estrogen Level in the Blood of the Study Subjects

Estrogen is an important sex hormone that has a role in many functions of the reproductive system [47]. It also has effect on various none reproductive activities in the human body, such as cardiovascular function, brain, and bone homeostasis [48]. Ovaries are the main source of estrogen in female and it can be synthesized in other none reproductive organs such as liver, muscle, bone, heart and brain [49]. Estrogen can associate with cancers of different types of organs of the human body such as mammary, ovaries, and endometrial tissues [50]. Breast cancer is the most associated type malignant disease with estrogen in females [51]. Levels of sex hormones in serum of premenopausal women were affected by the menstrual cycles when it is high in women with long cycles and less in those with short cycle [52]. On land-Moret *et al.* [53] found that postmenopausal women with high urinary excretion of estrogen and other androgens hormones are at high risk of breast cancer.

4.3 Residence, Family and Surgical History of Subjects

Breast cancer is affected by variable factors related to the family and the life style of the patients. It is considered a common malignance disease with four ranking among Iraqi females with an increasing in young females of Karbala province from 2009 to 2017 [1]. Results of this study showed that most patients with breast cancer living in urban area of the province of Karbala and a great number of them had a family history of this disease. From study of the relationship between breast cancer and a family history in Tabriz (Iran), it was found that family history and residence, especially in rural areas, were involved in the incidence of breast cancer and increased risk of this type of malignant disease [54]. Residence could be associated with the stage of the breast cancer. Although some evidences revealed a weak association between rural-urban residence and the stage of the breast cancer [55-56], the risk of late stage breast cancer increases in women residents in rural countries [57]. The African-American people living outside the city of Chicago are found to be at high risks of breast cancer with difficulty in early detection of the disease [58]. This was also observed among women living in rural countries of Metropolitan status of USA in which late breast cancer was more than 11% compared with their metropolitan or urban counterpart [59].

4.4 Determination of Candida spp. Counting in Involved Subjects

Vagina could be considered a suitable environment for different types of organisms, including bacteria, fungi and parasites. Yeasts represented by *Candida* species are one of the most residence organisms in the vagina of the lower genital tract which could be turned into pathogenic in specific conditions [60]. Vaginal contents of fungi still needed an illustration by more studies since a little is known about its living condition in vagina compared to bacterial community [61]. *C. albicans* is the most species of *Candida* spp. that have the ability to live as a mycobiota in the vagina [2].

5. Estrogen and Breast Cancer

A specific connection between estrogen and breast cancer has been studied for more than 100 years [56]. High blood level of estrogen in the patients with breast cancer is mostly demonstrated by many studies which proved estrogen as a risk factor for development of breast cancer, especially in postmenopausal women [60]. Estradiol (E2) is the most active type of estrogen associated with breast cancer development. Clemons and Gross [52] found several evidence supporting the hypothesis that estrogen and its metabolites are associated with the initiation and promotion of breast cancer in a complex matter. Catechol estrogen quinine 4 (4-CE) and catechol estrogen-3, 4-quinones as estrogen metabolites in the human body have a role in initiation of breast cancer through reacting with DNA to induce oncogenic mutation. The main mechanism of estrogen to cause breast cancer is usually starting from realizing of metabolites after its metabolism to play genotoxic and mutagenic activities resulting in stimulation of tissue growth [5]. These events usually work by the help of ER, especially ERα, in which binding of estrogen with it will activate oncogenic growth pathways in breast cancer cells [20]. Thus, genotoxic metabolites of estrogen, as with estradiol type, can work together with ERs for the development of breast cancer [5]. However, Yue et al. [62] introduced evidences to support two hypotheses about the role of estrogen in the development of breast cancer. The first hypothesis descries that estrogen increases cell proliferation through stimulating the transcription rate mediated by ER which may increase the genetic errors during DNA replication and leading to elevation of cancer development in breast tissue. The second hypothesis illustrates that qunione derivatives metabolite from estradiol can react with DNA leading to removing some of genetic bases from it by depuration process and the mutation chance will be increased during repairing of such error.

In addition to high level of estrogen, many endocrine-association factors can be considered risk factors for development of breast cancer, including high levels of androstenedione and testosterone hormones, converting androgens to the estrogenes (estrone and estradiol), and elevated urinary levels of estrogens and androgens [63]. Women with higher excretion of estrogen and androgens hormones through urine are considered at risk of breast cancer [64]. Postmenopausal women are another condition encouraging the effect of estrogen in development of breast cancer when the blood of those women has higher levels of estradiol [65]. Thus, measurement of sex hormones in postmenopausal women can be used as a predictable marker for breast cancer up to 16-20 years [66]. Moreover, concentration of estradiol and estrone is found higher in young women and in those with obese, smoking (15 cigarettes per day), and alcohol (20 g alcohol per day) [64].

Depending on the type of ER, breast cancer is divided starting from 1970 into two types: ER-positive and ER-negative [6]. The absence of ER in form of ER-negative may result from suppression of ER gene due to the DNA methylation in CpG island of the ER gene 5' region. However, ER-negative breast cancer is very active malignant type through its ability to increase the expression of the receptors of p53, CerbB2 and epidermal growth factor than it does by ER-positive breast cancer [67].

Patient with breast cancer usually showed a variable percentage in the presence of ER. ER-positive mostly presents a high rate of ER among patients with breast cancer compared with those with ER-negative [6]. This high rate of ER-positive breast cancer in California has no variable difference in the histological type of breast cancer, or its geographical distribution [68]. ER-positive is found as a major type in postmenopausal breast cancer patients (72%) than in premenopausal patients (57%) and the development of this type of ER-positive breast cancer in postmenopausal women can be increased after

treatment with some of estrogen therapies [69].

A viability of many factors can be associated with increasing the risk for development of ER-positive breast cancer such as nulliparity, delayed childbearing, early menarche, and postmenopausal obesity [70]. In the presence of ER-negative primary breast cancer, the risk for development of contralateral breast cancer can become higher compared with those with ER-positive type [71]. Races and age also have effect on the presence of ER types when white postmenopausal women at age 75-79 years have more ER-positive than black premenopausal women at age 50 years which had ER-negative type [72]. On the other hand, Anderson ET AL., (2002) found that white women had more ER-positive than black women at age 35-54 years and the rate of ER-negative can increase in age up to 50-54 years.

5.1 Estrogen Effects on Vagina and Vaginal Microbiome

Vagina can be affected by the estrogen level in the circulating blood depending on the stage of menstrual cycle in women. Decreasing level of estrogen during elderly age of women will lead to low estrogen level in vagina, while it became high in premenopausal stage, which has effects on the maturation of vaginal tissues, and returns again into low level during menopausal period [10].

The presence of ER in the vagina makes it affected by variable activities of estrogen [9]. Both of ERα and ERβ are found on the vaginal stroma and epithelial surface [73]. Basal, parabasal, and intermediate cell layer are the most enrichment area with ER in vagina epithelium tissues, while its location in stroma is mostly found in the vaginal lamina propria [74]. The concentrations of ER in vagina range between low level (4 fmol/mg protein (1 fmol = 10^{-15} mole)) and high level (119 fmol/mg protein). These concentrations are mostly affected by the site of ER and the period of menstrual cycle and not by the level of estrogen in the circulating system [75]. The concentration of ER in both of premenopausal and postmenopausal could be variable between low and high. Wiegerinck et al. [75] found that ER higher in postmenopausal women (4 to 119 fmol/mg) and low in premenopausal women (12 and 91 fmol/mg), while Di Carlo et al. [76] found insignificant differences in ER level between those two reproductive sexual stages (10-83 fmole/mg in postmenopausal and 12-78 fmole/mg in premenopausal women). Expression of ERα was found to reduce in vaginal mucosa and stroma of postmenopause stage in compared to those in premenopausal women, while ERβ expression reduced in the mucosa of postmenopausal women compared to those in premenopausal stage [77].

5.2 Estrogen Effect on Candida spp.

Estrogen as well as other steroid hormones has been proved to have direct multifunctional effects on various pathogenic microorganisms through regulation of microbial replication, colonization, biofilm formation and adhesion with host surfaces [78]. Its production and other factors such as disturbance of microbial balance, immune activity, can change vaginal physical environment and encourage development of vagina fungal infection [3]. A variety of *Candida* spp., especially *C. albicans*, can bind with estrogen by its content of a specific binding protein called estrogen binding protein (EBP1) [78]. EBP1 is mainly located in the nucleus of *C. albicans* and not in the cytoplasm [79]. Thus, *Candida* spp. has been shown *in vitro* and *in vivo* sensitivity to estrogen with a concentration depending matter [11]. Decreasing of estrogen level has encouragement role in development of vaginal candidiasis [80].

Several studies proved that the growth or colonization of *C. albicans* in vagina can increase in the presence of estrogen. This stimulation of growth could be increased 8.6-fold in vagina due to the effect of estradiol [11]. *C. albicans* is found to be survival and vital in the vagina of rat for up to 10 days after treatment with estradiol cypionate compared with

untreated group [81]. Adhesion of *C. albicans* on the vaginal epithelial tissue can also increase in the presence of estradiol or estriol with variable degrees [82].

Encouragement of estrogen to vaginal infection with *Candida* spp. can be explained by two mechanisms. The first is that estrogen has a direct effect on *Candida*spp. to grow fast through its contents of ERP1 [8]. 17β-estradiol and ethynyl estradiol at concentrations 10^{-5} to 10^{-10} M increase forming of germ tubes by *C. albicans* through their effect on increased expression of *CDR1* and *CDR2* genes, while this effect was low in the presence of 17α-estradiol or estriol [8].

The second mechanism of estrogen growth elevation of *Candida* spp. depends on the effect of estrogen to inhibit or attenuated of immunity defensive mechanisms in vagina [83]. Based on experiment in mice, inhibitory effect of vaginal epithelial cells against the growth of *C. albicans* was found to reduce in the presence of estrogen and any decreasing in estrogen level will encourage *C. albicans* to cause vaginal infection [83].

6. Correlation between Estrogen Level and *Candida* Species in the Vagina

Vagina is usually affected by the estrogen level in the blood stream and any change in this level can be noticed on the structure of vagina, especially during menstrual cycle [10]. Facilitation of estrogen effects on the vagina is performed through the presence of ER in the vaginal tissues [9]. In postmenopausal women, decrease of estrogen level leads to vaginal atropy, which is characterized by increasing dryness, low pH, and thinning tissue [10]. Thus, the occurring of *C. albicans* in vagina can be affected by the levels of estrogen and can be progressive to fungal infection [84]. VVA (vaginal or vulvovaginal atrophy) is the most common type of candidiasis that can be developed under the effects of lower level of estrogen [85].

7. Conclusions

The estrogen was found at normal level in most patients with ER-positive breast cancer and in healthy individuals, while its high level was higher among patients with ER-negative breast cancer. Age has no effect on the level of estrogen which could be increased at age over 30-39 years. Urban of Karbala province was the common residence of almost all involved subjects and a family history of breast cancer was recorded among two types of patients with breast cancer. Surgical history was observed among only patients with ER-positive breast cancer. *Candida* spp. was diagnosed with high counting in the vagina of patients with ER-positive breast cancer, while most of healthy individuals had absence of this yeast. Counting of *Candida* spp. was greater in subjects with normal level of estrogen, especially in those with ER-positive breast cancer. *C. albicans* was most commonly isolated species of *Candida* and its counting was greater in patients with ER-positive breast cancer and healthy individuals who had a normal level of estrogen. Counting of *C. albicans* was greater in patient with ER-negative breast cancer who had high level of estrogen.

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