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# Evaluation of Certain Physiological Biomarkers in Iraqi Endometrial Carcinoma Patients

**Hajer S. Hameed, Jabbar H. Yenzeel[1] & Majeed A. Sabbah[2]**
[1]Department of Biology, College of Science, University of Baghdad
[2]Forensic DNA for research and training center
***Corresponding author:** Hajer S. Hameed E-mail: hajar.sabah91@gmail.com, mobile: +9647727359776

## ABSTRACT

**Background:** Endometrial Cancer (EC) is the malignant tumor originating from endometrium cell (lining of the uterus). EC incidence and mortality have increased in recent years. Routinely used methods for EC diagnosis and treatment are histopathological tissue culture after surgery and postoperative radiotherapy, however there is still not enough efficient treatment for recurrence or progression of this disease. So, there is a critical need for further EC identification by new biological ways for the prognostic diagnosis of it.

**Objective:** This study aimed to look for ways by which could help in diagnosis of EC before the hysterectomy.

**Materials and Methods:** 55 patients with EC and 57 healthy women were involved in this study (up to 45 years) during their attendance at the Medicine City Hospital of Baghdad and Al-Yarmouk Hospital. The study was conducted from October 2020 to October 2021. Via vein puncture collection of blood samples from each women participated in this study for measurement of the levels of some proteins including Hepcidin hormone (hepc), Human epididymis protein-4 (HE-4), Transforming growth factor-beta (TGF-β) and CRP biomarker levels in serum of EC and healthy women by ELIZA method.

**Results:** This study showed a highly significant ($P \leq 0.01$) increase in TGF- β and HE4 in EC patients compared with healthy control. Hepc and CRP also revealed a highly significant ($P \leq 0.01$) increase in EC patients' women when compared to healthy control.

**Conclusion:** It can be concluded that TGF- β and HE-4 proteins could be used in the early diagnosis for EC. Addition of hepcidin and CRP estimation to TGF- β and HE-4 proteins results in accurate prognostic diagnosis.

**Keywords:** Endometrial cancer, Hepcidin, Transforming growth factor-β, Human epididymis protein- 4, C-reactive protein.

## INTRODUCTION

A common malignancy in female reproductive system is EC with an increased incidence [(1)]. EC is a tumor with heterogeneous texture. To enhance the prognosis of EC in patients by early, accurate, and effective diagnosis, the diagnosis is related to tumor size, grade and stage [(2)]. EC occurrence after menopause is most common. Hormones are key agents in sex-related cancers, such as EC; which is the 6$^{th}$ most prevalent cancer in women and poor prognostic indicators lead to their mortality [(3)]. In 2018, 380,000 new EC cases were noticed worldwide; in the UK, 9703 new cases were diagnosed between 2016 and 2018 [(4)]. Significantly, instance rates increased in countries with fast economic advancement, including Asian countries [(5)]. EC became the most prevalent gynecologic cancer in countries that under developed, which are responsible for almost 5% of cases of cancer and up to 2% of deaths by cancer in women [(4)].

Human epididymis protein-4 (HE-4) is a glycoprotein firstly discovered in the epithelial cells of the human epididymis. It was discovered by **Kirchoff *et al.*** [(6)] who's the first recognized HE-4 in males in distal epithelium of epididymis, which typically functions as proteinase inhibitor. It is under the family that inhibits serine protease (WAP domain proteins), which plays a function in carcinogenesis, tumor initiation and metastasis [(7)]. Based on identified activity, it is plausible that HE-4 plays an essential role in fertilization, specifically in maturation, motility and capacitation of sperm [(8)]. Although first distinguished HE-4 was in the reproductive tract of male, also it is present in other organs, like breast, kidney, lungs and reproductive tract of female [(9)]. HE-4 is a tumor marker of ovarian cancer, with 80% sensitivity [(8)].

Transforming growth factor β (TGF-β), a cytokine with a strong pleiotropy, which plays an important effect in cell proliferation, differentiation, migration, wound healing, apoptosis, adhesion, angiogenesis, immune surveillance and survival, angiogenesis, immunoregulation and cancer [(10)]. In *vitro* studies suggest that TGF β signaling regulates EC cell proliferation, survival and metastasis. Cells of the immune system produced TGF-β1 isoform, which exerts powerful activities as anti-inflammatory effect, and is a crucial regulator of immune response [(11)]. TGF-beta isoforms of (TGF-beta 1, 2 and 3) exist in mammals, which play key roles in regulation of growth and development of the cells [(12)]. Various cellular processes regulated by signaling pathway of TGF-β



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during homeostasis and during the development of tissue and organ [13].

Hepcidin hormone (Hepc) is protein that regulates entry of iron into the circulatory system. Hepc regulates iron metabolism by inhibiting the effect of its receptor ferroportin [14]. Hepc is predominantly synthesized by hepatocytes; also Hepc is expressed by malignant cells [15]. In many cancer patients, Hepc is elevated; but its expression level is low in other cells and tissues, including macrophages, adipocytes and brain [14]. Hepc possibly is crucial for the autocrine and paracrine control of iron fluxes and iron loading. Inflammation induces synthesis of Hepc, and is suppressed by erythropoiesis [15].

C-reactive protein (CRP) is an acute-phase protein of hepatic origin [16]. CRP is synthesised by the liver in response to factors released by macrophages and adipocytes, which increases interleukin-6 (IL-6) secretion by macrophages and T cells [17]. CRP is a plasma protein, its level in the circulatory system is increased in inflammatory response [16]. Its physiological effect is lysophosphatidylcholine binding, which found on the surface of dead, dying, cancer cells and some types of bacteria in order to stimulate the complement system [18]. This stimulation promotes phagocytosis by cells like macrophages, which eliminate necrotic cells, apoptotic, cancer cells and bacteria. Some organs in chronic inflammation show greater cancer risk [17]. An association is found between increased CRP levels and risk of cancer development. CRP elevation is an independent predictor for survival, as well as tumor recurrence [19].

## MATERIALS & METHODS

**Subjects:** One hundred twelve menopause and post-menopause women participate in this study with age 45- 65 years, 57 women with EC and 55 healthy women as a control. Their attendance to the Medical City in Baghdad, Baghdad Hospital in Medical City and Al-Yarmouk Hospital was during the period from October 2020 to October 2021.

**Collection of Blood samples and assay procedures:** Disposable syringes were used to draw blood samples from the participant's women via vein puncture, 5 ml was dispersal in gel tube, the samples were left about 40 min in room temperature, then centrifuged for 15 min at 3000 rpm to separate serum and stored in Eppendorf tubes at -20 º C until used for assay parameters. TGF- β and Hepc were measured by using ELISA (enzyme-linked immune-sorbent assay). HE-4 was measured by using cobas e 411 analyzer (Full automatic analyzer device) and CRP by Spin 200 (Full automatic biochemical device).

**Ethics approval:**

**Written informal consent was obtained from all patients and the study was approved by Ethical Committee. Ref.: CSEC/0122/0061, January 20, 2022, Department of Biology, College of Science, University of Baghdad. This study was carried out in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki) for studies involving humans.**

**Statistical analysis:**

The Statistical Analysis System- SAS (2012) program was used to effect the different factors in study parameters. T-Test was used for significance comparison between means.

## RESULTS

The results in table (1) showed highly significant ($P \leq 0.01$) increase of TGF- β and HE-4 concentration in women with EC (376.93 ± 13.35 and 284.51 ± 19.31 pmol/L respectively) as compared to healthy controls (47.04 ± 3.27 and 8.52 ± 0.63 pmol/L respectively). Statistical analyses of the results in table (1) also demonstrated a highly significant ($P \leq 0.01$) increase in Hepc hormone and CRP concentration in EC patients (299.36 ± 17.34 ng/ml and 44.72 ± 2.55 mg/L respectively) as compared to the healthy controls (72.95 ± 2.98 ng/ml and 2.52 ± 0.21 mg/L respectively).

**Table (1):** Serum markers and concentration in endometrial cancer patients and control

| Group | Mean ± SE | | | |
|---|---|---|---|---|
| | TGF- β (pg/ml) | HE4 (pmol/L) | Hepc (ng/ml) | CRP (mg/L) |
| Patients | 376.9± 13.35 | 284.51± 19.31 | 299.36± 17.34 | 44.72 ± 2.55 |
| Control | 47.04 ± 3.27 | 8.52 ± 0.63 | 72.95 ± 2.98 | 2.52 ± 0.21 |
| T-test | 26.39 ** | 36.93 ** | 33.71 ** | 4.887 ** |
| P-value | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

** ($P \leq 0.01$).

## DISCUSSION

The vital physiological processes, including proliferation, metabolism, differentiation, and apoptosis, are controlled by a powerful signaling network of cytokines that trigger, growth factors and/or polypeptide hormones [20]. The result of increase of

TGF-β in EC patients agrees with **Xiong *et al.*** [21]. TGF-β has been found to be overexpressed in certain cancer tissues, cancer cell lines and has also been well acknowledged as a biomarker predictor for various carcinomas [23]. In vitro studies suggest that TGF β signaling regulates EC cell proliferation, survival, and metastasis [20]. In recent years, there has increased focus on the effect of TGF-β signaling pathway in enhancing development of tumor and progression and promoting immunosuppression by tumor microenvironment (TME) [24]. TGF-β family regulates a huge array of biological activities where it promotes the transformation of fibroblasts [21] and regulates cell proliferation, differentiation, migration and apoptosis [25]. Also, TGF-β has an important role in development of embryo, immune responses, and in synthesis of extracellular matrix (ECM). Therefore, this pathway and disruptions play a key role in cancer suppression or promotion [22].

In cancer-associated fibroblasts (CAFs) and other stromal cells kinds, including inflammatory cells and endothelial cells. TME plays a key role in invasion and metastasis [12]. Biological processes of cancer progression also are regulated by CAFs in various mechanisms, such as affecting ECM remodeling [20]. CAFs are known to modify the ECM during progression of tumor, making it more permissive for tumor invasion into the surrounding tissue. TGF-β and specific interleukins are key secreted components for ECM remodeling [13]. Altogether, CAFs release soluble factors that contribute to enhanced migration and proliferation of EC, ultimately resulting in tumor progression [10]. ECM plays a key role in the female endometrium division in major remodeling changes every month [26]. Structure of ECM may regulate cell differentiation, proliferation, and the movement. By the activity of ECM-degrading proteases, for example, releases growth factors and cytokines that bound to matrix, and mediate the activation of downstream activities, such as various oncogenic transcription factors that plays role in EC. These factors that may encourage the EMT, that aid tumor cells in invasion and metastasize and are interesting targets for therapy in EC [27]. Expression of TGF-β from cancer cells supports cancer pathogenesis of tumor progression, which promotes anti-tumor immune evasion, metastasis and stemness [25]. A frequently noted example of the context-dependent roles is the dichotomy of TGF-β's roles in tumorigenesis [12]. TGF-β is a tumor suppressor for early-stage tumors and a potent growth inhibitor of cells of epithelial origin like EC [26]. But, in advance stage of cancers, tumor growth and progression promotes by inducing EMT and subsequent tumor invasion and metastasis [27].

Increase of HE-4 in EC patients agrees with **Han *et al.*** [28] who identified the expression of HE-4 in serum of EC and to explain links between expressions of HE-4, immunological parameters, and prognosis of disease [28]. HE-4 may be a tool for preoperative evaluation and postoperative surveillance of EC patients [29]. This result also agrees with **Behrouzi *et al.*** [30]. HE-4 overexpression in EC cell lines induced proliferation of cancer cell in *vivo* and in *vitro*, supporting that HE-4 has a tumor progression role [6]. This result also agrees with **Liu *et al.*** [31] who reported that HE-4 had a high rate of screening and high-risk factors for EC, early detection of HE-4 in serum may increase the diagnostic rate of EC that reduce range of missed diagnosis [9]. Numerous investigations have revealed that HE-4 is highly expressed in ovarian cancer tissue, as well as in other types of cancers including lung adenocarcinoma, pancreatic cancer and stomach cancer [30]. **Yang *et al.*** [32] used the immunohistochemical assay and ELISA to determine expression of HE-4 in tissue and peripheral blood from 31 cases of EC in the first time. Clinically, levels of HE-4 were higher in tissues with lymph node metastases [29]. HE-4's effect in normal and malignant tissue is still unclear. HE-4 is supposed to have a role in natural immunity [32]. However, HE-4 has myometrial invasion in EC [30]. Although, the biological function of HE-4 is unclear, overexpression of HE-4 activates several malignant phenotypes in the cells, including proliferation, invasion, independent growth, capability to anchorage and increase tumor growth in EC. Thus, the unregulated HE-4 levels observed in primary EC tissues may assist to EC progression [33].

The result of increase Hepc hormone in EC patients is in agreement with **Lelièvre *et al.*** [34] who reported that the level of Hepc increases in many cancers including endometrial cancer. This result also agrees with **Basuli *et al.*** [35]. Hepc is an important peptide hormone for the control efflux of iron and it contributes to the proliferation of cancer cells [14]. Moreover, the release and control of this hormone aren't regular within cancer tissues. High levels of hepc in cancer patients are due to inflammation. Liver secretes Hepc in response to iron loading and inflammation [34]. Numerous studies have shown that inflammations, obesity and cancers are associated with diminished iron status by increased Hepc levels [9]. The hepcidin–ferroportin axis contributes to the emergence of malignancies, particularly in the growth of tumors, angiogenesis and metastases [15]**.** In terms of their activity, cancer cells are quite active in metabolic requirements where they are in need to be supplied by important micro- and macronutrients for survival and proliferation [35]. Iron is one of the critical micronutrients, however iron can also participate in redox cycling and produce free radicals. [15]. Therefore,

iron can participate to both tumour initiation and growth. For DNA synthesis, iron is important where it is one of the causes of cell cycle arrest brought on by iron deficiency [34]. When malignant cells fed consistently subtoxic amounts of iron, they develop an aggressive phenotype that is prone to metastasis. [15]. Iron homeostasis disruption always impairs the functions of these iron-requiring proteins and is genetically associated with diseases characterized by DNA repair defects in mammals [14]. On the other hand, using iron-sequestering medications to starve cancer cells prevents tumor growth. Cancer cells must adjust to their increased metabolic need for iron by altering the expression of proteins involved in iron supply and iron export from cells [15]. Therefore, observing the variations in Hepc control between cancerous and non-cancerous cells is essential for understanding of survival and proliferation of tumor cells that help in discover new strategies to cancer treatment [34].

Increase of CRP in EC patients agrees with **Socha *et al.*** [17]. CRP can be combined with tumor markers to enhance the diagnostic efficacy toward EC [20]. On the other hand, higher pro-inflammatory cytokine CRP levels were associated with increased risk for EC [16]. Other inflammatory mediators such as TGF β and TNF α can increase CRP levels that is acute phase response, which occurs as a result of increasing IL-6 levels, which is produced by adipocytes and macrophages [20]. During inflammation, especially inflammation in acute phase, CRP supports T-cell activation and invasion into injured tissues, while the phase of chronic inflammation is mostly immunosuppressive [19]. Recent findings propose that epigenetic alterations in cancer cells are triggered by inflammation and composition of the TME [15]. These changes have the potential to influence and control many facets of development of cancer, including metabolic state, tumor growth, immune escape, metastatic spread and immunosupportive leukocyte generation [34]. Chronic inflammation affects epithelial cells and cancer cells, which perform modifications in histone and DNA methylation, suggesting that inflammation results in epigenetic alterations [18]. Scientists were explaining that there is a high relationship between inflammation and cancer mortality [17]. Worth mentioning is the fact that diagnosis of an early phase EC is more likely among postmenopausal women due to pain or an unexpected symptom as bleeding [32]. But not among menstruating women who also less likely would suffer from obesity, diabetes mellitus, etc. Therefore, EC detection by measurement of CRP in this group, which would gain the most from such a screening, could be more accurate [17].

## CONCLUSION

It can be concluded that TGF- β and HE-4 proteins could be used in the early diagnosis for EC. Addition of hepcidin and CRP estimation to TGF- β and HE-4 proteins results in accurate prognostic diagnosis.

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