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The Associations Between Hormones, Inflammatory Markers, And Blood Characteristics In Women With Abnormal Uterine Bleeding

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Abstract

Background: *Excessive or abnormal uterine bleeding AUB poses significant health challenges, specifically for perimenopausal women. Since AUB is a general clinical issue, identifying reliable biomarkers like COX-2 could address unmet medical requirements in identification and monitoring this problem.*

Materials and Methods: *Blood samples from 95 women (60 with abnormal uterine bleeding and 30 without AUB) were collected and examined for associations between commonly measured parameters, including hormone, antioxidant, interleukin-23, and cyclooxygenase concentrations, and abnormal uterine bleeding. The analyte concentrations were determined using ELISA assays.*

Results*: The current study display elevated levels of prolactin, interleukin-23, and cyclooxygenase in women with abnormal uterine bleeding compared to the control group. On the other hand, estradiol, progesterone, luteinizing hormone, and follicle-stimulating hormone levels were reduced. Interleukin-23 and cyclooxygenase-2 levels were significantly higher in the abnormal uterine bleeding group across different age and BMI ranges compared to controls.*

Conclusion: *Elevated levels of IL-23 and COX in women suffering from abnormal uterine bleeding might serve as prospective biomarkers to identify underlying disorders associated with AUB.*

Keywords*: Abnormal uterine bleeding, interleukin- 23, Cyclooxygenase-2,*

INTRODUCTION

Endometrial function is strongly controlled by hormonal signaling. Ovarian sex steroids estrogen and progesterone are fundamental for this regulation. Estrogen stimulates endometrial cell proliferation and regeneration, whereas progesterone opposes estrogen-stimulated proliferation and stimulates cell differentiation for implantation. Withdrawal of progesterone starts menstruation. Dysregulation of these routes can direct to abnormalities in endometrial breakdown, bleeding, repair, and regeneration [1]. Abnormal uterine bleeding (AUB), as well-defined by the International Federation of Gynecology and Obstetrics, involves a range of unusual menstrual signs stemming from several uterine abnormalities, usually displaying as irregular or heavy bleeding. Although infrequently life-threatening, the significances of abnormal uterine bleeding are substantial, as more than one-third of all women will encounter this condition at least once in their lifetime, often unpleasantly affecting their work efficiency and quality of life[2].

Abnormal uterine bleeding is a widespread issue associated to varied underlying causes, encompassing both structural abnormalities and functional disorders. Researchers have examined the potential contribution of inflammatory markers, such as interleukin-23, in the context of AUB. Alterations in the concentrations of circulating cytokines, including interleukins-10, 13, and 17, have been observed in women with uterine fibroids[3]. The serum concentration of cyclooxygenase-2 is linked to irregular uterine bleeding. Elevated COX-2 levels can affect the production of prostaglandins, which are involved in menstrual regulation and may contribute to abnormal uterine bleeding through mechanisms such as increased endometrial blood vessel formation and inflammation[4]. Studies have demonstrated that patients undergoing hormonal treatments like depot medroxyprogesterone acetate can effectively manage their irregular bleeding with selective COX-2 inhibitors like valdecoxib. suggest these inhibitors can give short-term control of irregular bleeding in this specific patient group[5]. Furthermore, research has shown that normal and endometrial cancer tissues exhibit significantly different patterns of COX-2 expression. Moreover, the endometrial adenocarcinoma cell line HEC-1B's growth and invasiveness were markedly inhibited by downregulating COX-2[6, 7].

Antioxidants may potentially play a role in managing abnormal uterine bleeding by reducing oxidative stress and inflammation, which are thought to contribute to various gynecological conditions. Oxidative

stress can disrupt hormonal pathways and uterine health, potentially impacting bleeding patterns. Heightened levels of reactive oxygen species have been linked to polycystic ovary syndrome, endometriosis, recurrent pregnancy loss, and other reproductive disorders [8, 9]. Accordingly, the relations between oxidative stress, inflammation mediated by COX-2, and their impacts on uterine bleeding patterns are significant for addressing and monitoring AUB effectively.

MATERIAL AND METHODS

Control and Patient Groups:

The study involved 30 control subjects and 60 patients, all between the ages of 35 and 50. All participants in this study were diagnosed with abnormal uterine bleeding. The medical history of each patient, including their age, duration of bleeding, history of hormonal therapy, and weight, was gathered. The participants in the control group were healthy and did not have any issues with their uteruses.

Blood Sample collection:

A blood sample volume of 5 ml was collected from both the patient and control groups. The collected sample amount was 3 ml of blood, which was placed in a gel tube. The sample was then centrifuged at 4500 RPM for 5 minutes to obtain blood serum for hematological testing. Afterward, the serum was frozen at -20 °C for screening of hormonal levels and serological tests.

Determination of Hormones, Antioxidants, and Interleukin-23:

The concentrations of hormones, antioxidants, interleukin-23, and cyclooxygenase were determined using the ELISA assay, following the kit guidelines, and the obtained concentrations were calculated based on the standard curve.

Statistical analysis:

Statistical analyses were performed using SPSS version 23.0. The results were reported as mean ± standard Error. An independent sample T-test was used to compare parameters across all studied groups, with corresponding p-values reported.

RESULTS AND DISCUSSION

The study findings showed that prolactin, interleukin-23, and Cyclooxygenase levels were elevated compared to the control group. In contrast, estradiol, progesterone, luteinizing hormone, and follicle-stimulating hormone levels decreased compared to the control group. Meanwhile, thyroid-stimulating hormones, antioxidants exhibited no significant differences between the study and control groups. Data are shown in tables (1-2).

Table (1): Serum level of female hormones among control and patient groups.

Parameters	Control	Patients	p-value
	Mean ±S.E.		
PRL	6.81±1.7	11.69±2.1	0.044*
E2	616.86±15.4	496.02±10.5	≤0.0001**
P	3.91±0.2	2.77±0.3	0.031*
TSH	1.82±0.1	1.64±0.4	0.665
LH	33.09±4.8	17.12±2.7	0.001**
FSH	30.55±6.2	11.90±1.8	≤0.0001**

Table (2): Serum level of interleukin-23 and antioxidants among control and patient groups.

Groups Parameters	Control	Patients	p-value
	Mean ±S.E.		
IL-23	55.26±4.7	205.11±10.7	≤0.0001**
Antioxidants	620.17±20.3	725.58±18.4	0.107
COX-2	0.59±0.02	1.05±0.03	≤0.0001**

One of the major causes of irregular uterine bleeding can be excessive prolactin. By inhibiting the release of gonadotropin-releasing hormone, hyperprolactinemia characterized by high prolactin levels which can disturb with the regular menstrual cycle and impact the synthesis of follicle-stimulating hormone and luteinizing hormone. In addition to triggering excessive or intermenstrual bleeding, this hormonal imbalance might initiate oligomenorrhea or amenorrhea, which are irregular or absent menstrual periods[10, 11].

Unusual menstrual bleeding may be caused by hormonal imbalances. Low progesterone consequences in unopposed estrogen, while excessive estradiol thickens the endometrium. Both of these factors can trigger heavy and irregular bleeding. The menstrual cycle is disturbed by differences in LH and FSH levels, which lead to irregular bleeding and anovulatory cycles[12].

20% of women of reproductive age suffer from abnormal uterine bleeding, which is a public condition that directs to the majority of hysterectomies. Its prevalence is linked with obesity due to modern lifestyles. AUB has a negative effect on quality of life. This is due to reasons such as anemia, the need for blood transfusions, effects on employment and sexual activity, as well as increased hospitalizations [13, 14]. In premenopausal patients, it can be challenging to specifically recognize the cause of abnormal or irregular uterine bleeding; however, pathological disorders of the endometrium or myometrium are commonly causing reasons. Anovulatory cycles are a frequent cause, but other options, such as hyperplasia, polyps, submucous myomas, and carcinoma, are additionally an alarm to the patients[14, 15]. Studies have combined uterine fibroids with infection and chronic inflammation, showing that inflammatory situations in the endometrium can contribute to their progress via processes such as enhanced extracellular matrix proteins and cytokine expression[16, 17]. One cytokine that is essential for inflammation and the immune response is IL-23. The expression of IL-23 in the human endometrium fluctuates with the menstrual cycle and pregnancy conditions and plays a role in regulating the endometrial immune reaction[18]. Previous studies have shown that people with and without endometriosis have significantly different serum levels of IL-1Ra, IL-23, and IL-37[19]. Elevated IL-23 levels may be a sign of a persistent uterine inflammatory process. Abnormal uterine bleeding may be caused by this inflammation, which can be linked to diseases like fibroids, endometriosis, or uterine infections.

Cyclooxygenase is a crucial enzyme in the biogenesis of prostanoids, which are signaling lipids that control distinct physiological routes, including inflammation, pain, renal function, and reproduction. COX catalyzes the transformation of arachidonic acid to prostaglandin H2, which is then converted to several prostanoids by specific synthases[20, 21].Growth factors and cytokines stimulate the COX-2 enzyme physiologically, and it is active at very low levels of arachidonic acid. Furthermore, COX-2 has been found to be overexpressed in several pathological conditions, including numerous cancers, where a poor prognosis is associated with its high expression, as well as other diseases related to inflammation, such as endometriosis[22]. Moreover, studies on humans and mice have shown how COX2 contributes to the onset and/or progression of a number of diseases[23].

Elevated cyclooxygenase levels, particularly COX-2, have been linked to increased production of prostaglandins, which can affect uterine contractility and lead to dysregulation in menstrual bleeding patterns. Peritoneal macrophages and endometriotic lesions are the key sources of the high levels of prostaglandin (PGs) observed in the peritoneal fluid of endometriosis patients. In addition, these patients have elevated levels of PGE2 because the estrogens, proinflammatory factors, and pro-angiogenic molecules in the peritoneal environment increase the expression of COX-2[22].

The level of the antioxidant has been shown to be higher in women with AUB mainly among those aged 35-41 in compared to the control group. As people age, oxidative stress increases, and antioxidants are essential in preventing this damage. Free radicals and antioxidants may become unbalanced as we age because our body's natural antioxidant defenses may deteriorate. This imbalance has the potential to harm cells and exacerbate age-related illnesses like cancer, neurodegenerative diseases, and cardiovascular diseases[24, 25].

Additionally, the result of the study showed that the levels of IL-23 were significantly higher in women with abnormal uterine bleeding compared to the control group, particularly among those aged 35-41. A similar trend was observed in the 24-28 age range, where the IL-23 values in the abnormal uterine bleeding group (207.60±18.4) were notably elevated compared to the control group (52.59±5.5). The same pattern

of increased IL-23 was also seen in the abnormal uterine bleeding cohort (161.24±6.7) relative to the control group (59.27±6.2). Data are shown in table 4. Aging weakens the immune function and upgrades chronic inflammation, leading to decreased the ability to fight diseases, as well as reduced wound healing and organ dysfunction[26]. Increased levels of IL-23 are linked with imbalances in the immune system and continual inflammation in women experiencing abnormal uterine bleeding. Younger women may actively produce IL-23 in response to inflammation, however as they age, their proficiency to regulate inflammation becomes less efficient, which might cause in decreased IL-23 production and the potential for inappropriate bleeding.

The serum level of COX-2 was elevated in women with AUB compared to the control group. This increase was observed across different age groups, with levels of 1.03±0.09 in the 35-41 age group, 0.98±0.02 in the 42-48 age group, and 1.32±0.16 in the 49-55 age group. Data are shown in table 4. Research shows that Cyclooxygenase-2 expression be likely to raise with age[27]. This enzyme is involved in inflammation and is often regulated in different age-related conditions, such as cancer. For instance, studies have presented that COX-2 expression is higher in older patients with conditions like colorectal cancer and papillary thyroid cancer[27].

According to body mass index (BMI), interleukin-23 levels were elevated in women with abnormal uterine bleeding who had a BMI within the 18-24.9 and 25-29.9 ranges, as well as in obese women, when compared to the control group (Data is shown in table 5). Previous studies suggested a significant correlation between obesity and increased IL-23 levels in plasma. The IL-23 concentration was notably higher, reaching 264.79, in the obese patient group. IL-23 levels and body mass index are significantly associated. According to studies, people with higher BMIs, particularly those who are obese, usually have higher IL-23 levels[28].

The level of antioxidant was found to be higher in obese patients compared to control group. The same tendency was noted with COX-2, which was raised across all BMI groups, with the highest levels absorbed in obese patients with abnormal uterine bleeding compared to the control group (Data is shown in table 5). The results of our study are aligned with previous research which indicates that people with a higher body mass index, specifically those considered obese, normally show an elevated level of the enzyme COX-2[29]. This is because Obesity is linked with chronic low-grade inflammation, and COX-2 is a significant enzyme involved in this inflammatory process.

Table (3): Serum level of Interleukin, Antioxidants and Cyclooxygenase components in patients and control groups according to age.

Groups / Parameters	Age (years)	Control	Patients	p-value
		Mean ±S.E.		
IL-23	G1	53.92±4.3	217.90±24.8	≤0.0001**
	G2	52.59±5.5	207.60±18.4	≤0.0001**
	G3	59.27±6.2	161.24±6.7	≤0.0001**
Antioxidants	G1	510.86±12.3	656.19±33.4	0.044*
	G2	710.40±15.4	786.59±16.7	0.648
	G3	639.26±20.3	702.86±20.4	0.712
COX-2	G1	0.54±0.12	1.03±0.09	≤0.0001**
	G2	0.58±0.03	0.98±0.02	≤0.0001**
	G3	0.64±0.08	1.32±0.16	≤0.0001**

-All results show significant differences(**G1**=age(**35-41**year), G2=age(**42-48**year), **G3**=age(**49-55**year).

Table (4): Serum level of Interleukin, Antioxidants and Cyclooxygenase components in patients and control groups according to the body mass index B.M.I.

Groups / Parameters	BMI	Control	Patients	p-value
		Mean ±S.E.		
IL-23	1	56.68±10.4	200.22±19.7	≤0.0001**
	2	55.00±9.9	144.23±16.4	≤0.0001**
	3	54.11±8.5	264.79±20.1	≤0.0001**

Antioxidants	**1**	643.89±20.3	750.24±25.2	**0.068**
	2	640.80±31.8	719.81±16.3	**0.102**
	3	575.83±16.7	706.12±13.4	**0.021***
COX-2	**1**	0.36±0.03	0.98±0.12	**≤0.0001****
	2	0.71±0.08	1.03±0.08	**≤0.0001****
	3	0.70±0.06	1.13±0.10	**≤0.0001****

-All results show significant differences (**1**=normal weight (**18-24.9**), **2**= overweight (**25-29.9**), **3**=obese (**>30**).

CONCLUSION

Higher levels of IL-23 and COX in women undergoing abnormal uterine bleeding could serve as biomarkers for identifying the basic issues contributing to AUB. Addressing the inflammatory approaches via anti-inflammatory medications or targeted treatments might assist in effectively managing the symptoms.

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