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Serum Levels of Immunological Factors Among Iraqi Women with Primary and Secondary Infertility

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ABSTRACT

Infertility, the inability to have a child after 1 year of unprotected intercourse, is a major health problem. The two varieties of infertility, primary and secondary are both prevalent. Aim of research: This study aimed to measure the levels of some immunological factors in Iraqi females with primary and secondary infertility and compare them with those in fertile women. The correlation between these factors was also investigated. Materials and Methods: This study was conducted on Al-Ramadi Teaching Hospital of Women and Children in Anbar, Iraq from 23 August 2023 to 31 December 2023. Ninety participants were recruited, including 60 infertile women (30 with primary infertility and 30 with secondary infertility) and 30 healthy women as a control group. All the participants were aged 18–45 years. Sandwich enzyme-linked immunosorbent assay was used to detect the blood serum level of adiponectin (ADP), visfatin, and resistin. For C-reactive protein (CRP), direct colorimetric method with kit provided from (GIESSE Diagnostics, Italy) was used. Results showed a significant increase in resistin ($P \leq 0.001$), ADP ($P \leq 0.01$) and CRP ($P \leq 0.0001$) levels in the serum of infertile women compared with those in fertile women. No statistical differences in visfatin were observed. Conclusion: ADP, resistin, and CRP were increased significantly in infertile women, and visfatin was not affected. The correlation among ADP, visfatin, resistin, and CRP was not significant.

Introduction

Infertility is a condition affecting the reproductive system of males and females [1]. It is the failure to conceive after 1 year of regular sexual intercourse without the use of contraception [2]. Numerous factors, including psychological, physical, and biological, can have an impact on this complex condition [3]. Worldwide, it is the third most prevalent condition after heart disease and cancer [4]. Infertility can lead to various negative effects on the psychological and social life of patients [5]. Localized statistics indicate that 10%–15% of couples wish to have a child but are not able to accomplish it. Globally, women seek treatment from various hospitals and fertility centers for primary and secondary infertility [6].

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Autoimmunity is a condition where self- and nonself-antigens cannot be distinguished by the immune system. Females more frequently develop autoimmunity than males. Embryo implantation is a crucial factor that affects female reproduction.

The immune system of women plays a significant role in the success or failure of pregnancy. It affects important steps, including ovulation and implantation. Therefore, autoimmunity is an essential factor to consider for females experiencing subfertility [7]. The link between female reproduction and autoimmunity has gained interest these days. Conception involves a series of intricate connections between the receptive uterus and the embryo [8]. These interactions are typically regulated by the immune and hormonal systems. In some cases, the ovary may be targeted by an autoimmune disorder due to systemic or organ-specific autoimmune conditions. This condition can result in

ovarian disorders, failures of implantation, and subfertility, all of which can affect a woman's ability to conceive [7].

White adipose tissues have multiple uses. Beyond their role as a store of energy, they also function as an endocrine organ. Adipokines secreted from adipose tissues should be examined to determine their impact on infertility. Secreted from adipocytes, adipokines—such as adiponectin (ADP), leptin, resistin, and visfatin—have diverse effects on the reproductive organs. Thus, assessing adipokines and sex hormones is imperative to further understand infertility and aid in its treatment [9]. ADP is a hormone containing 244 amino acids, and its molecular weight is 30 kDa. It inhibits the release of luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH) [10]. Although ADP receptors are found in gonadotrophs, somatotrophs, and thyrotrophin, ADP is primarily found in cells that produced growth hormone (GH), follicle-stimulating hormone (FSH), LH, and thyroid-stimulating hormone (TSH) [11].

Nicotinamide phosphoribosyl transferase (NPT), or visfatin (NAMPT), is another name for pre-B cell colony enhancing factor. It was first discovered to be a modulator of B cell differentiation expressed in the bone marrow, muscle, lymphocytes, liver, and fetal membranes [12]. Measuring 52 kDa, this protein has been recently renamed as visfatin because it is more prevalent in visceral fat than subcutaneous fat [9]. In healthy women of reproductive age, visfatin has a positive correlation with obesity and metabolic and hormonal abnormalities linked to polycystic ovary syndrome (PCOS) [13]. High levels of serum visfatin and low levels of ADP were found in women with PCOS [14], an important reason behind infertility [15]. Women with PCOS also have higher levels of visfatin gene expression than those without [16]. In the human ovary, visfatin impacts follicular growth, oocyte maturation, follicle dominance, selection, and ovulation [10]. It also plays a part in the regulation of human ovarian follicles [17].

Resistin is small and rich in cysteine. It is secreted as a polypeptide of 94 amino acids and was given this name because it imitates the mouse trait of insulin resistance adipokines. By contrast, human resistin is primarily secreted by peripheral blood mononuclear cells [10]. Diabetes and obesity are linked to increased resistin levels in diet-induced obesity [9]. Adipocytes from women with PCOS had a resistin gene overview, indicating that resistin might be involved in the local pathophysiology of infertility [18]. Resistin can obstruct insulin's function, resulting in insulin resistance; it weighs 12.5 kDa and is polypeptide released from rodents' adipocytes and human monocytes and macrophages [12].

Ovulation, embryo implantation, and ovarian folliculogenesis are dependent on immune cells and inflammatory molecules [18]. C-reactive protein (CRP) is a sensitive but nonspecific inflammatory indicator produced by the liver in response to different stimuli [19]. With interleukin 6 (IL6) having a monopoly on regulators [20] through its effects on the innate and adaptive immune systems and the complement system, CRP can modify the immune response and inflammation [21]. Some known risk factors for increased baseline hs-CRP levels that could lead to infertility include endometriosis, obesity, and PCOS [22]. Though low-grade inflammation is present in 20%–40% of reproductive-age women, CRP is not commonly used to diagnose or treat infertility [23].

Infertility in women increases the risk of chronic metabolic diseases, such as cardiovascular diseases. Hormonal changes, inflammation, and disrupted stress hormones all contribute to this risk [21].

Materials and Methods

Study Design

This study was conducted at Al-Ramadi Teaching Hospital for Women and Children in Anbar, Iraq from 23 August 2023 to 31 December 2023. Ninety participants were recruited, including 60 infertile women (30 with primary infertility and 30 with secondary infertility) and 30 healthy women (control group). All the participants were aged 18–45 years.

Inclusion Criteria

Healthy women were chosen using the following criteria: regular menstrual cycle (26–30 days), endocrine disorders have not been present in the past, and not using oral contraceptives or any drugs used to treat chronic conditions, such as diabetes or hypertension.

Ethical Approval

This study was confirmed by the approval committee of the University of Anbar Ethical under number of 128 in 11/12/2023.

Methods

Immunological analysis for adiponectin, visfatin, and resistin was performed using the sandwich-ELISA kit provided by Elabscience Biotechnology Co., Ltd., USA as shown in Fig (1–3). CRP was examined by direct colorimetry using the kit provided by GIESSE Diagnostics, Italy.

Statistical Analysis

IBM SPSS (version 28) and Gen Stat program were employed to detect treatment effect in study parameters. The results were analyzed statistically, and the parameters were represented by mean + S.E. Least significant difference) test or ANOVA was employed to compare the mean values. Pearson correlation was carried out for the studied traits. Statistical significance was considered at P≤0.05 and highly significant at P≤0.01. For graphics, the graph prism pad was used.

Results and Discussion

This study included 90 women (18–45 years old) divided in to three groups of 30: women with primary infertility (T1), women with secondary infertility (T2) and fertile women (Tc) as control. Other diseases affecting the variants were excluded.

Table.1: Variables in the study groups

	ADP	Visfatin	Resistin	CRP
Primary infertile women(T1)	6.65^{a}± 0.22	0.28^{a}± 0.1	77.4^{a}± 0.93	5.02^{a}± 0.34
Secondary infertile women(T2)	6.53^{a}± 0.27	0.28^{a}± 0.1	97.3^{b}± 0.98	1.92^{a}± 0.31
Fertile women(Tc)	5.29^{b}± 0.25	0.15^{a}± 0.08	58.8^{a}± 0.96	0.59^{a}± 0.03
LSD	0.97	N.S	18.9	0.43

Results showed that the serum ADP concentration in the women with primary and secondary infertility were significantly increased compared with those in the control group (6.65 ± 0.22, 6.53 ± 0.27, 5.29 ± 0.25) (P≤0.05) as shown in Fig (1).

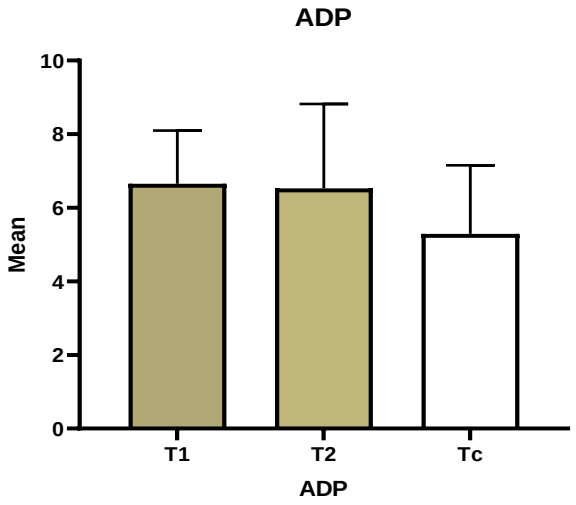

Fig (1) ADP concentration in women with primary (T1) and secondary (T2) infertility and control (Tc)

GnRH neurons are essential parts of the reproductive axis in the hypothalamus, regulating gonadotropin synthesis and release. In vitro research revealed that adiponectin activates adenosine monophosphate-activated protein kinase, which prevents hypothalamic cells from secreting GnRH [10]. In disagreement with our results, Natraja et al. [8] found no significant different in adiponectin levels between infertile women and control.

No statically significant difference in serum visfatin level was observed among the groups (T1: 0.28 ± 0.1 ng/mL, T2: 0.28 ± 0.1 ng/mL, and Tc: 0.15 ± 0.08 ng/mL). However, an increase in mean value was observed in the women with two types of infertility compared with that in the control as shown in Fig (2).

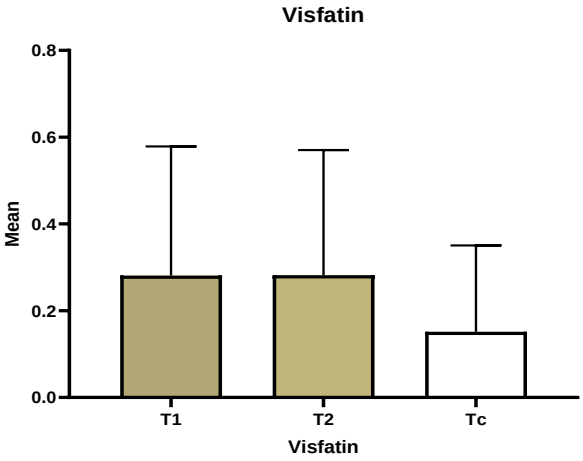

Fig (2 Visfatin concentration in women with primary (T1) and secondary (T2) infertility and control (Tc)

Visfatin is the cause of menstrual disorder and ovulatory infertility in women [24]. Visfatin and resistin elevated the phosphorylation of AMP in LbT2 cells and decreased LH secretion from LbT2 cells. Gonadotroph cells express visfatin and resistin and may impact female fertility by controlling LH secretion at the pituitary gland [25]. Our previous study [26] and Farshchian et al. [27] found no significant difference among infertile women, women with PCOS, and fertile women.

The results showed a significant increase in serum visfatin level in T2 (97.3 ± 0.24) compared with that in T1 and Tc (77.4 ± 0.93 and 58.8 ±0.08, respectively) ($p \leq 0.01$). No significant difference was found between T1 and Tc as shown in Fig (3).

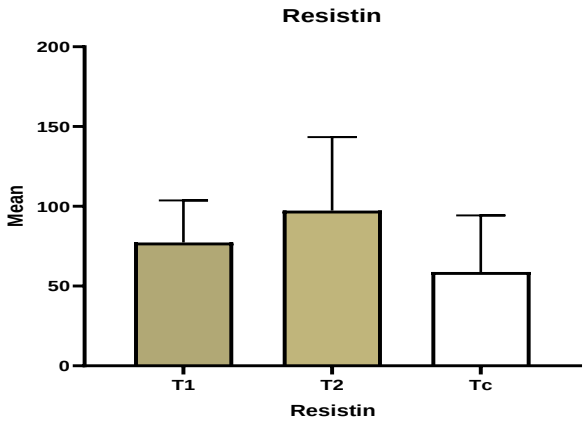

Fig (3) Means± SE of resistin in the serum of women with primary and secondary infertility and control

Inflammatory cytokines are suggested to play a role in the infertility of women with endometriosis (EM), and resistin levels are increased in women with EM [18]. Resistin level was significantly higher in infertile women with EM than in fertile women without EM [28]. Statistical results showed that the CRP concentration was elevated significantly in T1 compared with that in T2 and Tc. In addition, the level in T2 was elevated significantly compared with that in the control group (T1: 5.02 ± 0.34, T2: 1.92 ± 0.31, Tc: 0.59 ± 0.03) mg/dL ($P \leq 0.05$) as shown in Fig (4).

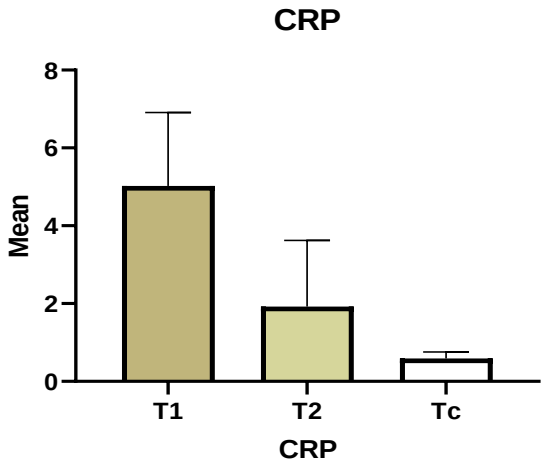

Fig (4) Means± SE of (CRP) in the serum of women with primary and secondary infertility and control.

Chronic inflammation is connected to several reproductive diseases associated with infertility, including PCOS and EM. **[**29**]** Ovarian inflammation has been directly linked to some causes of infertility **[**21**]**.

Our results concurred with the studies of Zhou et al. **[**30**]**, who stated that CRP was elevated significantly in infertile female compared with that in the control group, and **[**31**]** Zhang et al. **[**19], who proved that no significant difference in CRP serum level existed among women with primary and secondary infertility. Our findings also disagreed with Azarbayjani, AL-Lami, and colleague [32],[21], who found that the CRP level did not differ significantly between the case and control groups.

Table 2: Correlation among immunological factors in this study

	ADP	Visfatin	Resistin	CRP
ADP pearson Correlation	**1**	**-.022**	**.149**	**.053**
Visfatin pearson Correlation		**1**	**.084**	**-.062**
Resistin pearson Correlation			**1**	**.026**
CRP pearson Correlation				**1**

No significant correlation was found among ADP, visfatin, resistin, and CRP.

A previous study showed that sex hormones were negatively correlated with ADP and positively correlated with visfatin and resistin; these associations may play a role in the development of infertility

Conclusions

ADP and CRP levels were significantly high in women with both types of infertility, and resistin was elevated significantly in women with secondary infertility but not in women with the primary type. Visfatin was not affected by infertility. This study highlights the importance of considering resistin and ADP levels when diagnosing and treating infertility in women.

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Conflict of Interest

No conflicts of interest

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مستوى بعض العوامل المناعية في مصل النساء العراقيات المصابات بالعقم الاولي والثانوي

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الخلاصة :

العقم هو مشكلة صحية كبيرة ويعني العجز عن تحقيق الحمل بعد مرور 12 شهرا" او أكثر على ممارسة الجماع المنتظم وبدون وسائل حماية. وقد يكون العقم اوليا وهو عدم القدرة على الحمل مطلقا أوثانويا وهو عدم القدرة على الحمل مع وجود حمل مسبق. تهدف هذه الدراسة الى قياس مستوى بعض العوامل المناعية لدى النساء العراقيات المصابات بالعقم بنوعيه الابتدائي والثانوي ومقارنتها بالنساء الخصبات وكذلك التحقق من العلاقة الارتباطية لهـذه العوامل وقد اجريت هذه الدراسة في مستشفى الرمادي التعليمي للنساء والاطفال للفترة من 23اب الى 31كانون الاول 2023وشملت هذه الدراسة 90سيدة ،60منهن تعاني من العقم (30عقم اولي و30 عقم ثانوي) كما شملت 30امراة سليمة (مجموعة السيطرة) وكانت الاعمار بين 18–45عاما وتم اسـتخدام تقنية مقايسة الممتز المناعي المرتبط بالانزيم (الساندويش) لاجراء اختبارات الكشف عن كل من الاديبونكتين والريسستين والفسفاتين فيما تم استخدام القياس اللوني المباشر للبروتين التفاعلي حيث اظهرت النتائج وجود زيادة معنوية ذات دلالة احصائية لكل من الادبونكتين والرسستين والبروتين التفـاعلي فـي النساء المصابات بالعقم بنوعيه مقارنة بالنساء الخصبات كما لم تظهر فروقات ذات دلالة إحصائية في مستوى الفسفاتين في نتائج هذه الدراسة .كمـا تـم استخدام معامل ارتباط بيرسون للكشف عن الارتباط بين عوامل الدراسة حيث لم يظهر اي ارتباط معنوي بين كل من الاديبونكتين والرسستين والفسفاتين وكذلك البروتين المتفاعل.

الكلمات المفتاحيه: *العقم الاولي، العقم الثانوي، الادبونكتين، الرسستين، البروتين المتفاعل*