



MEASUREMENT OF WBCS, OBESTATIN AND B-LYMPHOCYTE ACTIVATING FACTOR LEVELS IN POSTMENOPAUSAL WOMEN WITH TYPE2 DIABETES

Suzan Najim Abdullah^{1*}, Ali Khudhyer Obayes² and Omar Thaer Jawad³

¹Pathological analysis dept. College of Applied Sciences ,Univ. of Samarra, Iraq

²Biology dept. College of Education ,Univ. of Samarra ,Iraq

*Corresponding author :Email:Suzan.najim94@gmail.com

Phone number :009647712123894

Abstract

The present study included the evaluation of WBCs count , Obestatin and B-lymphocyte activating factor (PAFF) among obese postmenopausal women with type 2 diabetes mellitus attending Samarra General Hospital and private clinics in Samarra City, Salah Al-Din Governorate. The study was conducted from mid-October 2022 to early February 2023. The study population comprised women aged between 55 and 85 years. A total of 120 women were enrolled in the study. Thirty apparently healthy women were included as the control group, while the remaining ninety participants constituted the patient groups.

White blood cell counts were assessed using a Mindray BS-5000 CBC analyzer. Serum levels of obestatin (OBST) and B-lymphocyte activating factor (BAFF) were measured using the enzyme-linked immunosorbent assay (ELISA) technique.

The results of the current study demonstrated a statistically significant increase in white blood cell counts and BAFF levels in the patient group compared with the control group ($p \leq 0.05$). In contrast, a statistically significant decrease in obestatin levels ($p \leq 0.05$) was observed in the patient group compared with the control group.

Key Words : WBCs, Obestatin ,PAFF, Obese women and DM2

1.Introduction :

Diabetes mellitus (DM) is a major public health concern, with a global prevalence of 9.3% (463 million individuals) in 2019, and it is projected to reach 10.2% in the coming years [1]. Obesity is one of the most important risk factors for diabetes, as it contributes to the development of insulin resistance, and it is more prevalent among women than men [2]. On average, women gain approximately 0.7 kg per year regardless of ethnicity, and the proportion of women who are overweight or obese increases after the age of 45 years [3].

During the postmenopausal period, weight gain and fat accumulation are primarily observed as a result of estradiol imbalance, which leads to dyslipidemia, insulin resistance, type 2 diabetes mellitus (T2DM), and cardiovascular diseases [4,5]. Increased visceral fat deposition enhances the production of adipokines, such as B-cell activating factor (BAFF), which is considered an important biomarker of inflammation [4]

Notably, quantitative and qualitative alterations in blood components, along with increased infiltration of immune cells, particularly neutrophils, into adipose tissue, represent key mechanisms underlying pathological immune activation during the onset and progression of obesity [6].

Hypertrophied adipocytes under insulin-resistant conditions contribute to disturbances in multiple lipid fractions, while simultaneously participating in the development of insulin resistance and the initiation of atherosclerosis [7]

Obestatin regulates several physiological functions, including appetite control, energy expenditure, gastrointestinal motility, gastric acid secretion, and insulin release [8]. It exerts its effects on the gastrointestinal tract, pancreas, adipose tissue, and cardiovascular system. Obestatin suppresses appetite and reduces food intake, and through its influence on cellular viability, it indirectly affects insulin secretion [9,10]

2.Materials and Method :

In this study, a total of 120 blood samples were collected during the period from October 15, 2022, to February 1, 2023. The study samples included ninety (90) obese females aged between 55 and 85 years who were diagnosed with morbid obesity and type 2 diabetes mellitus. These participants were attending Samarra General Hospital and private clinics in the city and had been clinically diagnosed by specialist physicians. In addition, thirty (30) apparently healthy women with no history of chronic diseases were included and considered as the control group.

3.Collection and preservations of blood samples:

Venous blood samples (5 mL) were obtained from both patients and control participants. Each sample was separated into two fractions. One milliliter was allocated for white blood cell analysis and collected in EDTA-K3 tubes. The tubes were gently agitated on a laboratory mixer for five minutes to allow adequate mixing with the anticoagulant. Thereafter, the samples were analyzed using a Mindray BS-5000 automated hematology analyzer, with results produced within two minutes.

The remaining four milliliters were placed in silica gel tubes to allow clot formation. Following centrifugation at 3000 rpm for five minutes, serum was carefully aspirated using a micropipette and preserved at -20°C until hormonal measurements were carried out

4.Levels of variables (Obestatin,PAFF) were estimated by using the Enzyme Linked ImmunoSorbant Assay (ELISA) kit from (Sunlong.China); This test measures the amount of the variables in blood serum using also the Enzyme Linked Immuno Sorbant Assay (ELISA) .

5.Bio-Statistical Analysis

The results were triplicated, expressed as mean $\pm$ SD due to results may be higher or lower than standard deviation. *T*-test was used by SPSS software on computer version 23 for assessment of the results. Significant variation was considered when the *P* value less than 0.05.

6.Results and Discussion :

6.1.WBCS count in study groups :

The results of the study showed that the mean $\pm$ standard deviation of total white blood cell count in the patient group was $10.05 \pm 2.75 \times 10^9/\text{L}$, whereas the control group recorded a value of $7.72 \pm 1.42 \times 10^9/\text{L}$, as illustrated in Figure (4-2). A statistically significant increase in total white blood cell count was observed in the patient group compared with the control group at a probability level of $P \leq 0.05$

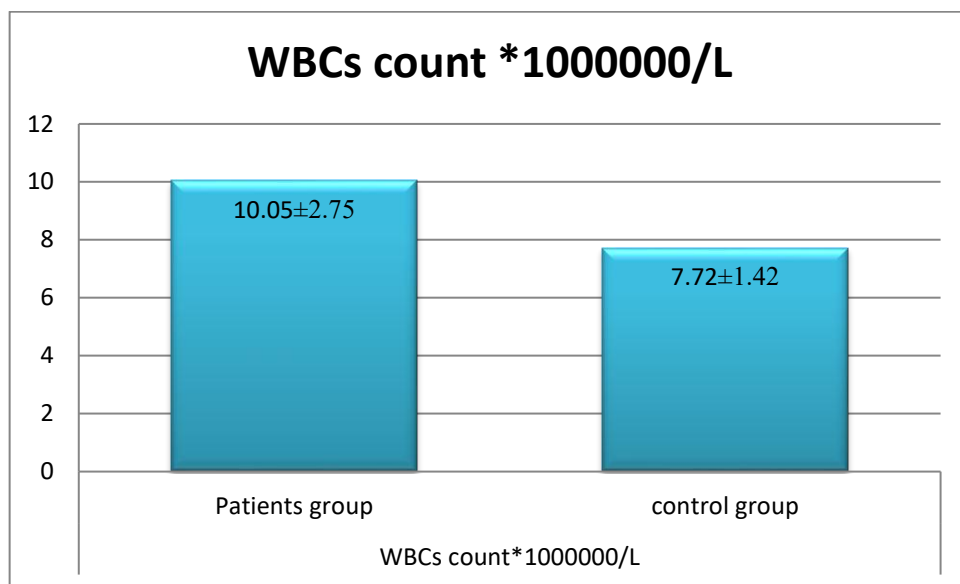


Fig.1 illustrates the levels of WBCs count in the patient group compared with the control group (mean ± standard deviation)

The findings of the present study are consistent with those reported by Kizilgul et al. [11] and Kheradmand et al. [12], who demonstrated elevated white blood cell counts in patients with diabetes compared with healthy control subjects. In contrast, the current results are not in agreement with those reported by Kashima [13], who found no significant association between white blood cell count and the incidence of diabetes among the Japanese population. However, other studies conducted in Japan have indicated that white blood cell count may serve as an independent risk factor for type 2 diabetes [13].

Several components of the complete blood count, including white blood cells, red blood cells, and platelets, are influenced by type 2 diabetes mellitus. Diabetes may lead to anemia, abnormalities in erythrocyte morphology and hemoglobin synthesis, as well as increases in white blood cell and platelet counts [14]. Elevated white blood cell counts have been associated with a higher risk of cardiovascular diseases in patients with diabetes [15,16], suggesting that these alterations may represent early indicators of cardiovascular complications. Accordingly, white blood cell count can be considered a practical and cost-effective tool for assessing cardiovascular risk in diabetic patients. Moreover, increased white blood cell counts have been linked to several cardiometabolic risk factors [14].

In addition, specific white blood cell subtypes have shown a strong association with glycated hemoglobin (HbA1c) levels, supporting the concept that diabetes mellitus is, at least in part, an inflammatory disorder [17,18]. Numerous studies have also identified white blood cell count as a marker of systemic inflammation, with a well-established relationship to insulin resistance and type 2 diabetes mellitus [19].

Given the close relationship between inflammation and diabetes, the identification of inflammatory markers may be essential for early detection of diabetic patients, prevention of chronic complications, and improved disease management. Measurement of white blood cell count is simple, widely available, and can be performed in virtually any clinical setting [20,21].

6.2.Levels of Obestatin in study groups :

The results indicated that the mean ± standard deviation of serum obestatin (OBST) levels in the patient group was 15.07 ± 3.73 pg/mL, whereas the control group exhibited a significantly higher level of 34.3 ± 10.9 pg/mL, as illustrated in Figure (2). A statistically significant reduction in obestatin levels was observed in the patient group compared with the control group ($P \leq 0.05$).

These findings are in agreement with previous studies conducted by Qi et al. [22] and Lipple et al. [23], which reported a significant decrease in serum obestatin levels among patients with type 2 diabetes mellitus

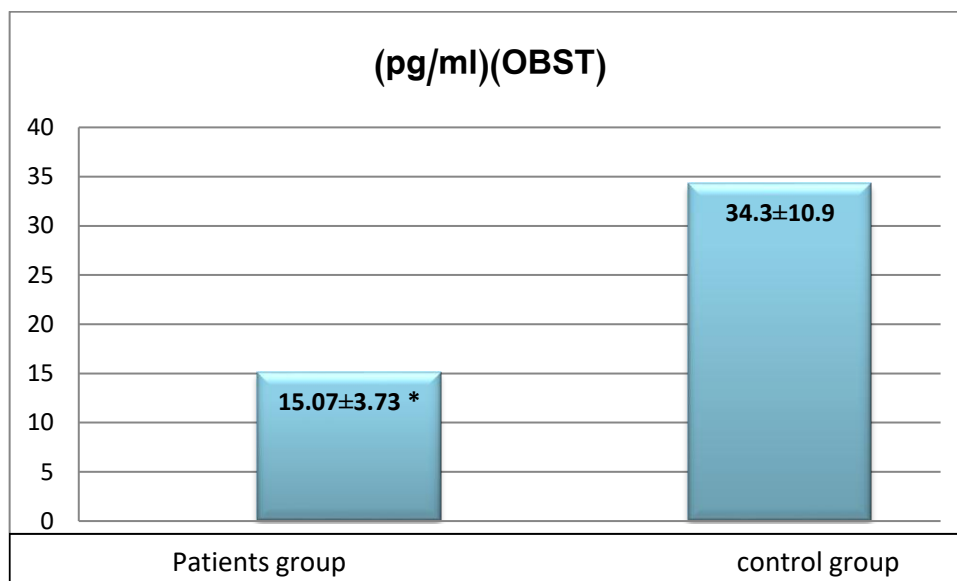


Fig.2 illustrates the levels of obestatin hormone in the patient group compared with the control group (mean $\pm$ standard deviation)

The observed reduction in obestatin levels among patients may be attributed to its role in appetite regulation or to the increased body mass index, particularly since the patient group consisted of obese women with type 2 diabetes mellitus. Previous studies have reported a negative correlation between obestatin levels and body mass index [24].

Several histological investigations have demonstrated that diabetes mellitus is associated with a reduced number of obestatin-producing cells in the gastric body, especially within the mucosal layer of affected individuals [25]. In addition, the hypothesis suggesting a possible interaction between obestatin and ghrelin has been supported, indicating that the balance between these two hormones may play a crucial role in body weight regulation [26].

Accordingly, obestatin appears to be involved in the regulation of food intake, body weight control, gastric motility, insulin biosynthesis, and the maintenance of pancreatic β -cell viability, as well as in the modulation of gene expression within these cells [27]. Furthermore, obestatin has been shown to inhibit lipolysis, reduce insulin resistance, suppress adipogenesis, and attenuate inflammatory processes. Through these mechanisms, obestatin may exert a therapeutic role in protecting against insulin resistance and various metabolic disturbances. Additionally, this hormone enhances the secretion of adiponectin, a key adipokine that improves insulin sensitivity by increasing energy expenditure and promoting fatty acid oxidation [28–30]

6.3.Level of B cell Activating factor(BAFF) in study groups :

The results indicated that the mean $\pm$ standard deviation of B-cell activating factor (BAFF) levels in the patient group was 188.6 ± 22.6 pg/mL, compared with 158 ± 21.4 pg/mL in the control group, as illustrated in Figure (3). A statistically significant increase in BAFF levels was observed in the patient group compared with the control group ($P \leq 0.05$).

The elevated levels of this factor may result from inflammation associated with type 2 diabetes in obese women included in the study. Obesity itself is known to enhance the likelihood of systemic inflammation. BAFF (CD257), a member of the tumor necrosis factor (TNF) family, has been identified as a key factor promoting B-cell growth and differentiation, which in turn increases immunoglobulin levels in the blood [31,32]. BAFF is primarily expressed by immune cells, including monocytes, macrophages, and dendritic cells [33], and it exerts its effects mainly through binding to TNF receptors.

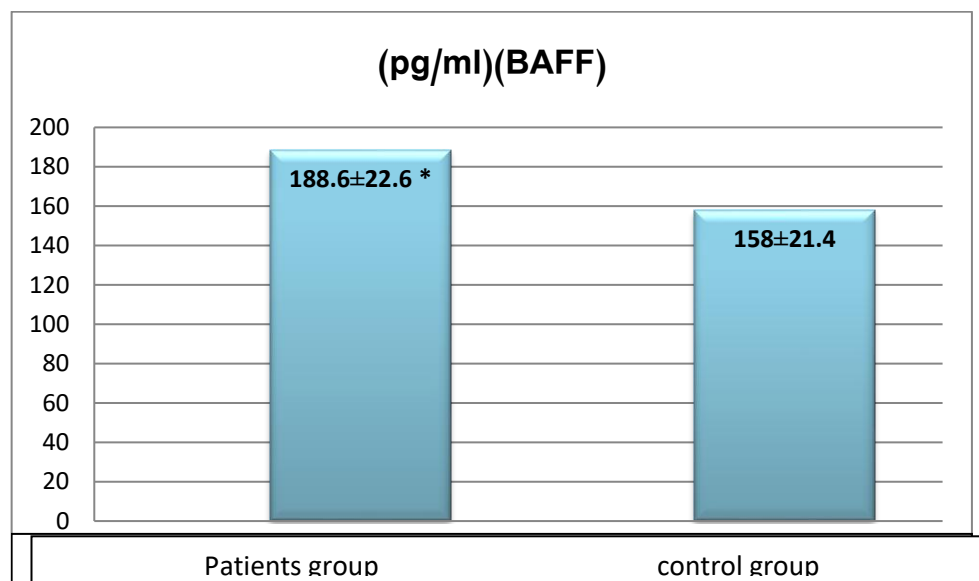


Fig.3 illustrates the levels of B-cell activating factor (BAFF) in the patient group compared with the control group (mean ± standard deviation).

Recent reports have revealed that BAFF is expressed in mature adipocytes and can promote adipogenesis in vitro [34]. This highlights the role of BAFF in inducing insulin resistance and suggests its significant contribution to increased inflammation in adipose tissue, which is associated with obesity, insulin resistance, and susceptibility to type 2 diabetes and metabolic syndrome [35].

Macrophages are recognized as a major source of BAFF secretion. Several studies have demonstrated that obese individuals exhibit an increased number of inflammatory cells, including macrophages and monocytes [36]. These findings indicate that macrophage-derived BAFF plays a specific role in promoting and accelerating insulin resistance, similar to the effects of TNF- α [37].

Moreover, alterations in lipid metabolism have been observed, including triglyceride accumulation, changes in lipid droplet size, and increased synthesis of free fatty acids and adipokines. These factors are strongly associated with obesity, metabolic syndrome, and diabetes [38]. Differentiated adipocytes secrete various bioactive molecules, such as TNF- α and BAFF, which are known to promote insulin resistance [39,40]. The production and secretion of BAFF by mature adipocytes suggest that this molecule functions similarly to certain adipokines

7.Conclusions: The observed elevation in B-cell activating factor indicates a clear interaction between the immune system and the pathological mechanisms of the disease, reflecting the potential contribution of immune responses to the development of functional disturbances. This supports the notion that immune alterations may be associated with the disease pathway, even if their direct causal role is not established. Similarly, the significant reduction in obestatin levels among patients with type 2 diabetes may reflect a disruption in metabolic regulation, particularly in obese patients, suggesting an imbalance in hormonal control related to obesity and insulin resistance

8.Acknowledgments

We extend my heartfelt appreciation to the medical laboratories for their invaluable cooperation and assistance throughout this study. Their expertise and support in conducting the necessary tests and providing accurate data were essential to the completion of this research. We are also profoundly thankful to all the participants who generously offered their time and effort, making this study possible. Their trust, patience, and willingness to contribute significantly enriched the quality and outcomes of this work.

9.Conflicts of Interest

The authors declares that there are no conflicts of interest related to this study

10. References :

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract.* 2019;157:107843.
2. American Diabetes Association. Standards of medical care in diabetes—2016. *Diabetes Care.* 2016;39(Suppl 1):S1–S112.
3. Karvonen-Gutierrez CA, Lee H, Lumeng CN. Body weight changes in women through midlife: The role of menopause. *Maturitas.* 2016;85:1–9.
4. Sinatora RD, Varghese M, et al. Postmenopausal adiposity and metabolic risk: Role of estradiol imbalance and adipokines. *J Endocrinol Invest.* 2022;45(3):451–463.
5. Varghese M, Sinatora RD, et al. Visceral fat, inflammation, and cardiovascular risk in postmenopausal women. *Clin Chim Acta.* 2020;501:210–218.
6. Daryabor G, Atkin SL, et al. Obesity-induced immune dysregulation: Role of neutrophils in adipose tissue inflammation. *Obes Rev.* 2019;20(6):850–867.
7. Blaszczak A, et al. Hypertrophied adipocytes and lipid metabolism in insulin resistance and atherosclerosis. *Metabolism.* 2021;119:154740.
8. Lin L, et al. Obestatin: Physiological roles in appetite, gastrointestinal motility, and energy balance. *Peptides.* 2014;56:53–59.
9. Villarreal R, et al. Obestatin's impact on β -cell viability and insulin secretion: Mechanistic insights. *Endocrinology.* 2022;163(2):bqac001.
10. Pradhan G, et al. Regulation of insulin release by obestatin: Implications for metabolic control. *J Endocrinol.* 2017;232(2):123–134.
11. Kizilgul M, et al. White blood cell counts in diabetic patients: A comparative study with healthy controls. *Diabetes Res Clin Pract.* 2018;140:123–130.
12. Kheradmand M, et al. Hematological changes in type 2 diabetes mellitus: Clinical implications. *J Diabetes Metab Disord.* 2019;18(2):345–352.
13. Kashima Y. Association of white blood cell count with type 2 diabetes in a Japanese population. *J Diabetes Investig.* 2019;10(5):1200–1207.
14. Barbieri M, et al. Hematological alterations in diabetes mellitus: Implications for metabolic and cardiovascular risk. *Curr Diabetes Rev.* 2015;11(3):175–184.
15. Tong PCY, et al. White blood cell count and cardiovascular risk in type 2 diabetes. *Diabetes Care.* 2004;27(5):116–121.
16. Wheeler T, et al. Hematologic predictors of cardiovascular events in diabetes mellitus. *Clin Exp Hypertens.* 2004;26(6):465–476.
17. Milosevic I, Panin L. Inflammatory status and leukocyte subtypes in type 2 diabetes. *Clin Immunol.* 2019;208:108244.
18. Sorokina E, et al. Leukocyte subpopulations and HbA1c in patients with type 2 diabetes. *Endocrine Connections.* 2021;10(6):789–798.
19. Du T, et al. White blood cells as markers of systemic inflammation and insulin resistance in type 2 diabetes. *Diabetologia.* 2009;52:164–171.
20. Zhang X, et al. Inflammatory markers and diabetes: Clinical significance of WBC measurement. *J Clin Lab Anal.* 2017;31(4):e22045.
21. Tahir A. White blood cell count as a simple tool for assessing cardiovascular and metabolic risk in diabetes. *Diabetes Metab Syndr.* 2015;9(2):101–105.
22. Qi Y, et al. Serum obestatin levels in patients with type 2 diabetes mellitus: A comparative study. *Metabolism.* 2007;56(10):1416–1420.
23. Lippl F, et al. Reduced circulating obestatin concentrations in type 2 diabetic patients: Implications for metabolic regulation. *J Clin Endocrinol Metab.* 2008;93(8):3145–3150.
24. Cuiping Y, Dongmei L. Relationship between circulating obestatin levels and body mass index in type 2 diabetes. *Clin Chim Acta.* 2009;401(1–2):108–112.

25. Gao X, et al. Histological analysis of obestatin-producing cells in the gastric mucosa of diabetic patients. *Peptides*. 2010;31(9):1620–1625.
26. Bounias M, et al. Interaction between obestatin and ghrelin: Implications for body weight regulation. *Endocrine*. 2022;76(2):301–310.
27. Fujimiya M, et al. Physiological roles of obestatin in food intake, insulin biosynthesis, and β -cell gene expression. *J Endocrinol*. 2012;215(1):45–54.
28. Rovella V, et al. Obestatin enhances adiponectin secretion and improves insulin sensitivity. *Metabolism*. 2021;120:154794.
29. Granata R, et al. Obestatin modulates energy expenditure and fatty acid oxidation in metabolic disorders. *J Clin Endocrinol Metab*. 2012;97(11):E2139–E2147.
30. Ren Y, et al. The role of obestatin in metabolic regulation: Effects on adiponectin and insulin sensitivity. *Diabetes Obes Metab*. 2008;10(12):1167–1174.
31. Qiao J, et al. Role of B-cell activating factor (BAFF) in B-cell proliferation and differentiation. *Immunology*. 2017;152(3):457–467.
32. Ajash A, et al. BAFF-mediated regulation of immunoglobulin production in inflammatory conditions. *Cytokine*. 2018;104:1–8.
33. Liu Y, et al. Expression of BAFF in immune cells: Monocytes, macrophages, and dendritic cells. *Front Immunol*. 2018;9:1117
34. Swaroop JJ, et al. BAFF expression in mature adipocytes and its role in adipogenesis in vitro. *J Cell Biochem*. 2012;113(4):1234–1242.
35. Oleinika K, et al. BAFF as a mediator of inflammation in adipose tissue: Implications for insulin resistance and metabolic syndrome. *Metabolism*. 2022;133:155169.
36. Kim J, et al. Increased inflammatory cells in obese individuals: Focus on macrophages and monocytes. *Obesity (Silver Spring)*. 2008;16(11):2432–2440.
37. Rotter V, et al. Macrophage-derived BAFF and its contribution to insulin resistance: Similarity to TNF- α effects. *Cytokine*. 2003;24(3):118–125.
38. Tchoukalova YD, et al. Lipid metabolism alterations in obesity: Triglyceride accumulation, free fatty acid synthesis, and adipokines. *Obesity (Silver Spring)*. 2010;18(12):2337–2344.
39. Kopp A, et al. Differentiated adipocytes secrete TNF- α and BAFF: Implications for insulin resistance. *J Biol Chem*. 2009;284(7):4233–4241.
40. Tilg H, Moschen AR. Adipokines and insulin resistance: The role of BAFF and TNF- α . *Nat Rev Endocrinol*. 2008;4(9):441–449.