Journal of Pioneering Medical Sciences
Received: May 12, 2025 | Accepted: July 22, 2025 | Published: August 05, 2025
Volume 14, Issue 07, Pages 01-06

DOI https://doi.org/10.47310/jpms2025140701

Evaluation of ANGPTL8 and TGF-β1 Levels in Type 1 Diabetes Patients: Association with Gender and Age

Zainab A. Fadhil[1*]
[1]Department of Biology, College of Education for Pure Science, University Diyala, Iraq

Author Designation: [1]Associate Lecturer

*Corresponding author: Zainab A. Fadhil (Zainab.a.fadhil@uodiyala.edu.iq).

Abstract **Background:** The common chronic autoimmune disease known as type 1 diabetes (T1D) results in insulin insufficiency because it promotes the death of pancreatic β-cells through inflammation. This study sought to assess the immunological markers Angiopoietin-like protein 8 (ANGPTL8) and Transforming Growth Factor Beta 1 (TGF-β1) levels in Iraqi patients with type 1 diabetes in comparison to healthy controls, as well as any correlations with age and gender. **Material:** There were two groups of patients in the case control study: a healthy group of 12 women and 28 men, and a group of 27 females and 23 males with T1D. In accordance with their ages, the participants were split into three groups: Group I consisted of people aged 20 to 39, Group II of people aged 40 to 59, and Group III of those aged 60 to 80. The latex agglutination test was used to assess CRP, and the levels of ANGPTL8 and TGF-β1 were determined using the ELISA technique. **Results:** The findings demonstrated that patients' TGF-β1 concentrations were comparatively higher 18.82±4.49 ng/mL than those of healthy individuals 17.45±3.77 ng/mL. In contrast to the healthy control 519.71±18.90 ng/mL, the ANGPTL8 protein's concentration in patients was somewhat lower 509.53±34.7. There was a significant difference in ANGPTL8 levels between the age groups of 20-39, 0-59, and 60-80 years; male ANGPTL8 levels were found to be higher and to decline with age. TGF-β1 declined in women as they aged, but it rose in men. **Conclusion**: Age and gender are important confounding variables for TGF-β1 and ANGPTL8 levels in T1D. When evaluating these biomarkers or creating future T1D predicting research, these demographic factors need to be taken into consideration.

Key Words Angiopoietin-Like Protein 8, Tgfβ1, C-Reactive Protein, T 1d

INTRODUCTION

Insulin-secreting pancreatic β-cells are destroyed by T-cells in type 1 diabetes, an autoimmune disease. When the body's own β-cells fail, people need to take insulin from outside sources to control their metabolism and survive [1]. Although T1D can develop at any stage of life, cases noticeably rise throughout puberty. As T1D's age of onset increases over time, it becomes more difficult to distinguish it from type 2 diabetes, especially in people who have a genetic predisposition. Genes linked to the human leukocyte antigen (HLA) are the main risk factor [2-3].

198 amino acids make up the 22 kDa protein called angiopoietin-like protein 8. The gene responsible for ANGPTL8 will be found on chromosome 19p12.2. Mostly found in the liver, white fat, and brown fat, this protein is essential for glucose homeostasis, lipid transport, and the body's response to chronic inflammation. The high blood levels of ANGPTL8 have been associated with a number of medical conditions, including metabolic syndrome, type 2 diabetes, atherosclerosis, hypertension, and non-alcoholic fatty liver disease or non-alcoholic steatohepatitis [4]. ANGPTL8, also known as betatrophin, plays a crucial role in the metabolism of fats and carbohydrates in people with type 1 diabetes. Research indicates that individuals with chronic type 1 diabetes have elevated levels of ANGPTL8 in their blood, which may be linked to the disease's advancement and consequences such as dyslipidemia and coronary artery disease (CAD) (X6). The control of lipoprotein lipase (LPL), an enzyme crucial to triglyceride metabolism, depends on ANGPTL8. Higher lipid levels in patients with type 1 diabetes have been linked to dyslipidemia, which is defined by abnormal blood lipid levels [5]. Prior research has demonstrated that ANGPTL8 is a key player in the regulation of inflammation. It has been demonstrated that when pro-inflammatory cytokines like

TNF-α activate it, more matrix metalloproteinases and other inflammatory cytokines are produced, which causes tissue inflammation [6].

Growth Factor Transformation A crucial cytokine, beta-1 (TGF-β1) influences a number of cellular functions, including growth, differentiation, and death. High levels of TGF-β1 can exacerbate inflammation and aid in the autoimmune attack on pancreatic β-cells in Type 1 Diabetes (T1D) [7]. TGF-β1 is mostly produced by NK cells, white blood cells, and dendritic cells [8]. There are three different isoforms of TGF-β: TGF-β1 to TGF-β3, with TGF-β1 being the most recognized and researched. Through serine/threonine kinase receptors, TGF-β primarily transforms growth factor beta receptors 1 (TGFBR1), 2, and 3 [9]. The involvement of TGF-β1 in type 1 diabetes is complicated. It is well-known for its immunosuppressive qualities, which aid in tolerance maintenance and may shield against autoimmune reactions directed at pancreatic β cells [10]. In mice, islet-specific overexpression of TGF-β1 eliminated autoimmune reactions and corrected the diabetes phenotype. TGF-β1 has been studied as a therapeutic drug to reduce autoimmunity against β cells [11]. But because of its function in controlling immunological responses, it may also have an impact on the course of disease by affecting regulatory T cells and other immune systems [12].

The purpose of this research is to examine how age and gender affect ANGPTL8 and TGF-β levels, as well as how TGF-1bate and circulating ANGPTL8 concentrations relate to each other in Iraqi individuals with type 1 diabetes. Although the relationship between blood ANGPTL8 levels and TGF-β in diabetic patients has been the subject of numerous investigations, type 1 diabetics have received very less attention.

METHODS

Patients and Control

Blood samples were taken from 50 T1D patients and 40 healthy individuals during the September 2024-January 2025 research period. after being identified in accordance with the World Health Organization's guidelines for diagnosing diabetes by a specialized doctor in the consulting clinic at Muqdadiyah General Hospital in the Diyala Governorate, Iraq (WHO, 2011). There are 23 men and 27 women with T1D.. The control group for this study included 28 healthy male and 12 female participants. The age range of the patient and healthy research groups is the same, ranging from 17 to 20 years. Venous blood withdrawal was used to obtain the samples, and wine plastic medical syringes were used to extract 5 ml of blood. After being computed, the blood was put in test gel tubes and left to coagulate for half an hour at room temperature. Centrifugation was then used to separate the serums for five minutes at 3000 rpm. In Eppendroff, the serum was separated into equal quantities (250 microliters). We kept the preserved serum at -20°C until we needed it, to prevent frequent thawing and freezing. You should only use each piece once.

Exclusion Criteria

All patients with type 2 diabetes melliuts, gestational diabetes mellitus, pancreatitis, cancer of pancreas, patients with renal impairment, liver disorder and pregnant women also have been excluded.

Determination the Concentration of Angptl8

The sandwich enzyme linked immune sorbent assay is based on technology. 96-well plates had already been coated with the anti-ANGPTL8 antibody. Additionally, antibodies are detected using the biotin-conjugated anti-ANGPTL8 antibody. After adding the test samples, standards, and biotin-conjugated detection antibodies to the wells, wash buffer was used to rinse them. After adding horse reddish peroxidase streptavadin, unbound conjugates were removed using wash buffer. HRP enzymatic processes have been modeled using TMB substrate. TMB was catalyzed by HRP to produce a blue product that became yellow when an acidic stop solution was added. The amount of material that ANGPTL8 captures on the plate is reflected in the yellow color density. The concentration of ANGPTL8 can be computed by measuring the O.D. absorbance at 450 nm in a microplate reader.

Determination the Concentration of Tgf-B1

To test TGF-β1, the sandwich ELISA technique was used. The human antibody TGF-β1 is applied to the plate beforehand. After being introduced to the sample, TGF-β1 attached itself to the well-coated antibodies. The material was then treated with biotinylated human TGF-β1 antibody, which bound to TGF-β1. Following its addition, Streptavadin-HRP attached itself to the antibody Biotinylated TGF-β1. Following unbound incubation, the streptavadin-HRP was removed during a washing phase. After applying the substrate solution, color was generated in accordance to the amount of human TGF-β1. Following the addition of the stop solution (an acidic solution), the absorbance at 450 nm was measured.

Statistical Analysis

The computer capability with the statistical programs of SPSS 23 were used to analyze the data. ANOVA was used to assess the significance of a mean difference between more than two groups, and the student t-test for independent samples was used to test the mean difference between two groups. The data was given in simple measures of number, mean, and SD. The significance level was set at a P value of equal to or less than 0.05. Additionally, Pearson correlation was used to assess the importance of the relationship between two quantitative variables.

RESULTS

Descriptive Characteristics of the Study Groups

Forty healthy control volunteers and fifty patients were recruited for this investigation. The demographic details of the patients and control participants are shown in Table 1.

Table 1: Demographic Characteristics of Patients and Healthy Control Subjects

Characteristic	Patients (n = 50)	Control (n = 40)	P
Age (years)	46.96±8.06	41.72±7.08	0.083
Sex			
Male	23 (46.0%)	26 (65.0%)	0.072
Female	27 (54.0%)	14 (35.0%)	
CRP			
Positive	30 (60.0%)	3 (7.5%)	<0.001
Negative	20 (40.0%)	37 (92.5%)	
TGF-β1	18.82±4.49	17.45±3.77	0.585
ANGPTL8	509.53±34.7	519.71±18.90	0.811

Table 2: The Demographic, Clinical and Laboratory Data for Female and Male Patients

Characteristic	Male n = 23	Female n = 27	P
Age	41.47±8.96	51.62±10.11	0.109
CRP			
Positive	17 (73.9%)	13 (48.1%)	0.064
Negative	6 (26.1%)	14 (51.9%)	
TGF-β1	20.36±4.21	17.5±4.75	0.426
ANGPTL8	569.03±52.23	458.86±45.13	0.015

n: Number of cases, SD: Standard deviation, †: Independent samples t-test, NS: Not significant at p>0.05

Table 3: Clinical And Laboratory Data for Aged Subjects

Characteristic	20-39 years (G1)	40-59 years (G2)	60-80 years (G3)	P
Male CRP				
Positive	6 (60.0%)	9 (81.8%)	2 (100.0%)	0.356
Negative	4 (40.0%)	2 (18.2%)	0	
TGF-β1	15.41±4.36	23.57±4.16	27.41±7.56	0.227
ANGPTL8	713.3±73.3[a]	436.88±60.1	436.3±48.8[c]	0.022*
Female CRP				
Positive	1 (33.3%)	8 (53.3%)	4 (44.4%)	0.789
Negative	2 (66.7%)	7 (46.7%)	5 (55.6%)	
TGF-β1	18.78±3.6	17.73±4.5	16.71±4.27	0.968
ANGPTL8	583.3±41.8	478.3±62.6	384.9±50.3	0.414

A: Indicate significance between G1 and G2 groups. b: Indicate significance between G1 and G3 groups. C: indicate significance between G2 and G3 groups

Table 4: Correlation Analysis between Angptl8 Levels and Tgf-B1 Biomarker of Revealed Interesting Gender-Specific Differences

Characteristic	ANGPTL8 Male n = 23		ANGPTL8 female n = 27	
	r	P	r	P
TGF-β1	0.510	0.001*	0.198	0.322

r: Correlation coefficient

The average age of the control participants was 41.72±7.08 years, whereas the average age of the patients was 46.96±8.06 years. The groups did not differ significantly from one another (p = 0.083). Gender-wise, there were 23 (46.0%) male cases and 27 (54.0%) female cases in the patient group and 26 (65.0%) male cases and 14 (35.0%) female cases in the control group. There was no significant difference in the frequency distribution of patients and control subjects by gender (p = 0.072). In comparison to control groups, patients exhibited a non-significant small drop (p<0.05) in ANGPTL8 (509.53±34.7) versus (519.71±18.90). Table 1 provides a summary of other characteristics of the sick and healthy subject groups.

Laboratory and Clinical Characteristics in Patients According to Sex

There were fifty patients in the study-twenty-seven women and twenty-three males. The patients' clinical, laboratory, and demographic data are displayed in Table 2. Male and female blood levels of ANGPTL8 differed considerably (mean of 569.03±52.23 vs. 458.86±45.13; p = 0.115). Male and female TGF-B1 levels, CRP, and age did not differ significantly, though.

ANGPTL8 Levels and Clinical Parameters According to Age

The levels of ANGPTL8 and other clinical markers for each age group under study are displayed in Table 3. It was discovered that while ANGPTL8 levels were non-significantly higher in men and increased with age, there was a significant difference in male ANGPTL8 levels between the age categories of 20-39, 0-59, and 60-80. TGF-β1 and CRP did not significantly differ between male groups (p<0.05), according to Table 2. TGF-β1 and CRP levels in the female groups did not differ significantly (p<0.05). TGF-β1 and CRP, however, did not differ significantly across the sexes.

Correlation Analysis between Angptl8 Levels and Tgf-B1 Biomarker of Revealed Interesting Gender-Specific Differences

Although no significant correlation was observed between ANGPTL8 and TGF-β1 in females. In contrast, male ANGPTL8 levels exhibited a significant positive correlation with TGF-β1 levels (Table 4).

DISCUSSION

The study's findings demonstrated that transforming growth factor β1 (TGF-β1) mean levels were greater in type 1 diabetic patients than in healthy people. Given that TGF-β1 is thought to be a major contributor to the inflammatory response linked to autoimmune disorders like type 1 diabetes, these findings are in line with earlier research [13]. Nevertheless, there was no statistically significant difference in TGF-β1 levels between the two groups, indicating that this increase might not be consistent or early in all patients. Numerous factors, such as a limited sample size, significant patient variation, or varying times after disease diagnosis, could be responsible for this finding [14]. The role of TGF-β1 in autoimmune disorders is complicated. It may play a role in the development of long-term diabetes problems including diabetic nephropathy and aids in the regulation of inflammatory responses [15]. Consequently, disease progression may have an impact on its concentration, which could account for the lack of substantial differences in this investigation [16]. However, prior research has demonstrated that TGF-β1 can be influenced by glycemic control, treatment type (e.g., insulin), genetic, and environmental factors, which increases the heterogeneity of outcomes among studies [16].

According to our research, type 1 diabetic patients had lower ANGPTL8 levels than healthy controls, however this difference was not statistically significant. Though not always with obvious statistical significance, these findings are in line with earlier research that suggested ANGPTL8 levels may be lowered in type 1 diabetes as a result of immune system alterations and metabolic abnormalities [17]. It has been discovered that ANGPTL8 plays a part in the control of glucose and lipid metabolism, which contributes to the pathophysiology of diabetes. It is anticipated that insulin-dependent conditions such type 1 diabetes will have an impact on its concentration. [5]. However, compared to type 2 diabetes, which is linked to relative hyperinsulinemia, ANGPTL8 alterations may be less noticeable in type 1 diabetes since the disease is characterized by a total or almost complete loss of insulin production as a result of immune-mediated β-cell death. This explains why our investigation found no significant differences [18]. This study supported the findings of another study in Kuwait's Arab adolescent population, which found that girls had lower ANGPTL8 levels than boys, indicating a gender difference in ANGPTL8 levels (2023). Genetic and environmental factors may influence ANGPTL8 levels, which could explain the variations across different populations. [19].

However, compared to type 2 diabetes, which is linked to relative hyperinsulinemia, ANGPTL8 alterations may be less noticeable in type 1 diabetes since the disease is characterized by a total or almost complete loss of insulin production as a result of immune-mediated β-cell death. This explains why our investigation found no significant differences [18]. This study supported the findings of another study in Kuwait's Arab adolescent population, which found that girls had lower ANGPTL8 levels than boys, indicating a gender difference in ANGPTL8 levels (2023). Genetic and environmental factors may influence ANGPTL8 levels, which could explain the variations across different populations. [20].

Males may have a higher expression of ANGPTL8 because of sex-specific fat distribution. Compared to females, who store body fat in their subcutaneous depots, males typically have higher visceral fat mass, which is associated with higher serum levels of the hormone [21-22]. This finding highlights the importance of considering sex when evaluating type 1 diabetes indicators. Because males and females have different insulin and fat sensitivity, it also opens the door for new therapeutic prospects where therapies might be tailored to ANGPLT8 in a sex-specific way [23]. In addition to encouraging the secretion of insulin by pancreatic beta cells and shielding them from apoptosis, estrogen also plays a crucial role in upregulating the liver's reaction to insulin. [24-25].

Our investigation revealed that ANGPTL8 levels were non-significantly higher in men and increased with age, but that there was a significant difference in ANGPTL8 levels between the age groups of 20-39, 0-59, and 60-80 years in males. Conversely, ANGPTL8 levels in female groups rose with age but did not differ significantly overall. Fat builds up in VAT as people age, increasing their risk of developing metabolic disorders [26]. ANGPTL8 did not seem to have a significant impact on glucose metabolism or pancreatic beta cell proliferation in models of insulin resistance brought on by high-fat diet feeding [27]. According to one study, the intra-abdominal fat area and central fat depot in postmenopausal women are 49% and 36% higher, respectively, than in premenopausal women [28].

In type 1 diabetes (T1D), the link between ANGPTL8 and TGF-β1 is complex and influenced by sex relationships, particularly between male and female individuals. Genetic, environmental, and physiological factors are some of the mediators that have been identified as responsible for the difference between the two groups [29]. looked into the male:female ratio of T1D and discovered that it is not just seen in people with immune-mediated diabetes or people with HLA-DQ. Their findings suggest that age has a significant role in the ratio and that male and female patients may exhibit different illness behaviors. This could imply that the processes, such as TGF-β1 and ANGPTL8, may also be different in the sexes.Additionally, efforts in [30] on the lung function of male T1D patients supports the physiological consequences of diabetes in men, which may be mediated via ANGPTL8 and TGF-β1-related metabolic and inflammatory pathways. The results also suggest that these indicators may be associated with particular problems in male patients [31]. who examined OS problems in male and female T1D rats further highlights the significance of sex in diabetic complications. Their findings indicate that sex differences significantly effect the impact of problems, which may be attributed to variations in TGF-β1 and ANGPTL8 levels.A study conducted by [32] specifically examined the expression of TGF-β1 in the early stages of type 1 diabetes mellitus fracture healing. They suggested that diabetes may prevent normal healing by suppressing TGF-β1 signaling, and they showed that the expression of TGF-β1 response to inflammation was considerably worse in diabetic participants than in controls at each time point following surgery.There has also been research on the role of TGF-β1 in diabetic nephropathy, a common consequence of diabetes. [33] specifically examined the expression of TGF-β1 in the early stages of type 1 diabetes mellitus fracture healing. They suggested that diabetes may prevent normal healing by suppressing TGF-β1 signaling, and they showed that the expression of TGF-β1 response to inflammation was considerably worse in diabetic participants than in controls at each time point following surgery.There has also been research on the role of TGF-β1 in diabetic nephropathy, a common consequence of diabetes. [34] also showed a negative association between miR-192 and TGF-β1 in patients with diabetic nephropathy, indicating that miR-192 may regulate the production of TGF-β1 and associated fibrotic processes. This highlights the subtleties of the TGF-β1 program in relation to diabetes and as a

target for treatment. Age and gender are two demographic factors that have been shown to affect TGF-β1. [35] and controlled studies examining TGF-β1 expression in individuals with lengthy bone fractures (depending on age and gender) may aid in elucidating the function of TGF-β1 in male patients with type 1 diabetes.

CONCLUSIONS

The study discovered that whereas women's TGF-β1 levels decreased with age, men's increased. A positive and statistically significant association between TGF-β1 and ANGPTL8 suggested a possible shared regulatory pathway, however ANGPTL8 levels decreased with age in both genders. Therefore, sex differences may be related to hormones, notably the aging-related decrease in testosterone levels in men, which is associated with higher levels of cytokines and increased low-grade inflammation. On the other hand, estrogen has an anti-inflammatory effect and can inhibit the expression of TGF-β1 and ANGPTL8 in women, particularly before and soon after menopause.

REFERENCES

[1] Abdulabbas, Dhifaf A. and Ekhlas Abdallah Hassan. "A case-control study to evaluate irisin levels in patients with type 1 diabetes mellitus." *Irish Journal of Medical Science (1971 -)*, vol. 193, no. 3, February 2024, pp. 1275-1282. http://dx.doi.org/10.1007/s11845-024-03626-4.

[2] Lefta, Rami Fadhel and Ekhlas Abdallah Hassan. "Serum soluble α-klotho levels in patients with diabetic nephropathy." *Irish Journal of Medical Science (1971 -)*, vol. 193, no. 2, August 2023, pp. 725-731. http://dx.doi.org/10.1007/s11845-023-03502-7.

[3] Abed, Baydaa Ahmed *et al.* "Role of stanniocalcin-1 and proenkephalin-a as novel biomarkers in prediction of newly diagnosed type 2 diabetic patients." *International Journal of Diabetes in Developing Countries*, vol. 45, no. 2, May 2024, pp. 488-494. http://dx.doi.org/10.1007/s13410-024-01353-1.

[4] Abu-Farha, Mohamed *et al.* "The multi-faces of angptl8 in health and disease: Novel functions beyond lipoprotein lipase modulation." *Progress in Lipid Research*, vol. 80, November 2020. http://dx.doi.org/10.1016/j.plipres.2020.101067.

[5] Li, C. *et al.* Factors influencing ANGPTL8 levels in metabolic diseases. *Endocrine Research*, vol. 4, no. 3, 2019, pp. 145-153.

[6] Abu-Farha, Mohamed *et al.* "The proinflammatory role of angptl8 r59w variant in modulating inflammation through nf-κb signaling pathway under tnfα stimulation." *Cells*, vol. 12, no. 21, November 2023, pp. 2563-0. http://dx.doi.org/10.3390/cells12212563.

[7] Bazzaz, Javad Tavakkoly *et al.* "Tgf-β1 and igf-i gene variations in type 1 diabetes microangiopathic complications." *Journal of Diabetes & Metabolic Disorders*, vol. 13, no. 1, April 2014, pp. 1-9. http://dx.doi.org/10.1186/2251-6581-13-45.

[8] Zorena, Katarzyna *et al.* "Serum concentrations of transforming growth factor-beta 1 in predicting the occurrence of diabetic retinopathy in juvenile patients with type 1 diabetes mellitus." *Journal of Diabetes Research*, vol. 2013, no. 1, 2013. http://dx.doi.org/10.1155/2013/614908.

[9] Chang, Albert S. *et al.* "Transforming growth factor-β1 and diabetic nephropathy." *American Journal of Physiology-Renal Physiology*, vol. 310, no. 8, April 2016, pp. F689-F696. http://dx.doi.org/10.1152/ajprenal.00502.2015.

[10] Ma, X. *et al.* The Expression of miR-192 and its significance in diabetic nephropathy patients with different urine albumin creatinine ratio. *Journal of diabetes research*, vol. 2016, no. 1, 2016, 6789402.

[11] Phillips, Brett E. *et al.* "Arrest in the progression of type 1 diabetes at the mid-stage of insulitic autoimmunity using an autoantigen-decorated all-trans retinoic acid and transforming growth factor beta-1 single microparticle formulation." *Frontiers in Immunology*, vol. 12, March 2021. http://dx.doi.org/10.3389/fimmu.2021.586220.

[12] Zhong, Ting *et al.* "Tgf-β-mediated crosstalk between tigit+ tregs and cd226+cd8+ t cells in the progression and remission of type 1 diabetes." *Nature Communications,* vol. 15, no. 1, October 2024. http://dx.doi.org/10.1038/s41467-024-53264-8.

[13] Chang, Albert S. *et al.* "Transforming growth factor-β1 and diabetic nephropathy." *American Journal of Physiology-Renal Physiology*, vol. 310, no. 8, April 2016, pp. F689-F696. http://dx.doi.org/10.1152/ajprenal.00502.2015.

[14] C. C., Möller *et al.* Transforming growth factor-beta and diabetic nephropathy: From molecular mechanisms to clinical implications. *Current Diabetes Reports*, vol. 19, no. 7, 2019, pp. 29.

[15] Frangogiannis, Nikolaos G. "Transforming growth factor-β in tissue fibrosis." *Journal of Experimental Medicine*, vol. 217, no. 3, February 2020, pp. 1286-1292. http://dx.doi.org/10.1084/jem.20190103.

[16] Yadav, H. *et al.* Transforming growth factor-beta1 promotes diabetes-induced endothelial dysfunction through differential regulation of nitric oxide and reactive oxygen species. *Journal of Biological Chemistry*, vol. 290, no. 49, 2015, pp. 30306-30319.

[17] Yin, Yanhua *et al.* "Increased serum angptl8 concentrations in patients with prediabetes and type 2 diabetes." *Journal of Diabetes Research*, vol. 2017, 2017, pp. 1-7. http://dx.doi.org/10.1155/2017/8293207.

[18] Wang, Y. *et al.* Role of ANGPTL8 in glucose homeostasis and lipid metabolism. *Diabetes and Metabolism Journal*, vol. 41, no. 4, 2017, pp. 261-271.

[19] Hassan, Ekhlas Abdallah, and Wafaa Sh Al - Zuhairi. "The impact of gender on the relation of serum angiopoietin-like protein 8 (angptl8) level with metabolic parameters in adults." *Obesity Medicine*, vol. 40, June 2023. http://dx.doi.org/10.1016/j.obmed.2023.100494.

[20] Xu, Fangfang *et al.* "Angptl8/betatrophin improves glucose tolerance in older mice and metabolomic analysis reveals its role in insulin resistance in hepg2 cells." *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy,* vol. Volume 14, October 2021, pp. 4209-4221. http://dx.doi.org/10.2147/dmso.s330700.

[21] Nogueiras, Ruben *et al.* "The central melanocortin system directly controls peripheral lipid metabolism." *Journal of Clinical Investigation,* vol. 117, no. 11, November 2007, pp. 3475-3488. http://dx.doi.org/10.1172/jci31743.

[22] Hassan, Ekhlas Abdallah *et al.* "Impacts of androgen abuse and overtraining on endocrine profile in bodybuilders." *Iraqi Journal of Science,* vol. 62, no. 12, December 2021, pp. 4614-4620. http://dx.doi.org/10.24996/ijs.2021.62.12.2.

[23] Mauvais-Jarvis, Franck. "Sex differences in metabolic homeostasis, diabetes, and obesity." *Biology of Sex Differences,* vol. 6, no. 1, September 2015. http://dx.doi.org/10.1186/s13293-015-0033-y.

[24] "Supar is a bad omen for chronic kidney disease progression." *Indian Journal of Forensic Medicine and Toxicology,* vol. 14, no. 2, April 2020, pp. 1523-1530. http://dx.doi.org/10.37506/ijfmt.v14i2.3141

[25] Khaleel, F.M. *et al.* "Evaluation of serum zinc in women of childbearing age and its relationship with obesity", *Journal of Medicinal and Pharmaceutical Chemistry Research*, vol. 4, no. 10, 2022, pp. 950-955.

[26] Hassan, Ekhlas Abdallah. "The relation between homocysteine, oxidative stress and atherosclerosis disease." *Indian Journal of Public Health Research and Development,* vol. 10, no. 7, 2019, pp. 537-0. http://dx.doi.org/10.5958/0976-5506.2019.01626.7.

[27] Wang, Y. *et al.* Role of ANGPTL8 in glucose homeostasis and lipid metabolism. *Diabetes and Metabolism Journal*, vol. 41, no. 4, 2017, pp. 261-271.

[28] Kodoth, Varna *et al.* "Adverse changes in body composition during the menopausal transition and relation to cardiovascular risk: A contemporary review." *Women's Health Reports,* vol. 3, no. 1, June 2022, pp. 573-581. http://dx.doi.org/10.1089/whr.2021.0119.

[29] Weets, I. *et al.* "Male-to-female excess in diabetes diagnosed in early adulthood is not specific for the immune-mediated form nor is it HLA-dq restricted: Possible relation to increased body mass index." *Diabetologia,* vol. 44, no. 1, January 2001, pp. 40-47. http://dx.doi.org/10.1007/s001250051578.

[30] Arif, Khan Mohammad *et al.* "Fvc, fev1 and fev1/fvc% in type-1 diabetic male and their relationships with hba1c." *Journal of Bangladesh Society of Physiologist,* vol. 7, no. 1, July 2012, pp. 23-28. http://dx.doi.org /10.3329/jbsp.v7i1.11156.

[31] Purushothaman, Indira *et al.* "Ocular surface complications result from dysregulation of the ogf-ogfr signaling pathway in female diabetic rats." *Experimental and Therapeutic Medicine,* vol. 22, no. 1, April 2021. http://dx.doi.org/ 10.3892/etm.2021.10119.

[32] Xu, M.T. *et al.* "Diabetes mellitus affects the biomechanical function of the callus and the expression of tgf-beta1 and bmp2 in an early stage of fracture healing." FapUNIFESP (SciELO), *Brazilian Journal of Medical and Biological Research,* vol. 49, no. 1, 2016. http://dx.doi.org/10.1590/ 1414-431x20154736

[33] Cristovam, Priscila C *et al.* "Role of chymase in diabetic nephropathy." *Experimental Biology and Medicine,* vol. 237, no. 8, August 2012, pp. 985-992. http://dx.doi.org/ 10.1258/ebm.2012.011356.

[34] Ma, Xiaoyu *et al.* "The expression of mir-192 and its significance in diabetic nephropathy patients with different urine albumin creatinine ratio." *Journal of Diabetes Research,* no. 1, 2016. http://dx.doi.org/10.1155/2016/6789 402.

[35] Kaiser, Georg *et al.* "Is the expression of transforming growth factor-beta1 after fracture of long bones solely influenced by the healing process?" *International Orthopaedics,* vol. 36, no. 10, May 2012, pp. 2173-2179. http://dx.doi.org/10.1007/s00264-012-1575-9.Reference Two