ORIGINAL PAPERS

Inflammatory Effect of Leptin and C-reactive Protein with Vitamin D Deficiency in Type 2 Diabetes Mellitus

Sanhareeb Wajdi Sabih AL-ROBAIEE[1], Sura Zahim HUSSEIN[2], Ahmed Sami FARHAN[2]

Abstract

Background and objectives: Diabetes is a complex metabolic disorder affecting the glucose status of the human body. Immune system activation is highly related to type 2 diabetes incidence, progression, adaptive and innate immunity is involved in the inflammation. Vitamin D insufficiency causes insulin resistance as well as glucose intolerance, which are symptoms of significant vitamin D deficiency. Increased inflammatory biomarkers and insulin resistance are prominent symptoms of this illness. **Materials and methods:** Serum specimens were taken from 120 people, with 70 patients (35 males and 35 females) with type 2 diabetic individuals and 50 healthy controls (25 males and 25 females) divided into two groups. Patients in the study population ranged from (18-50) years old and were hospitalized at Ballad and Salah Aldeen General Hospitals in Salah Aldeen province from August 2019 to February 2020. **Results:** Vitamin D levels were considerably reduced in females and male diabetic patients compared to controls, while leptin levels were elevated with ALP levels that were slightly diminished in diabetic males compared to controls but higher in diabetic females. Both C-reactive protein and serum uric acid levels were significantly higher in male and female diabetic patients compared to controls. In addition, these were increased in male diabetic individuals when compared to female diabetic individuals. **Conclusion:** In type 2 diabetes individuals, severe vitamin D deficiency and increased leptin cause inflammation and complicate bone metabolic illness.

Keywords: Diabetes mellitus, Vitamin D, leptin, C-reactive protein.

[1]Ballad General Hospital, Salah Aldeen Health Directorate, Tikrit, Iraq
[2]Clinical Biochemistry Unit, Salah Aldeen Health Directorate, Tikrit, Iraq

Corresponding author:
Sura Zahim HUSSEIN, Yarmook Street, Box 45 / Tikrit 28001 / Iraq
E-mail: dr.sura2012@yahoo.com

INTRODUCTION

Diabetes mellitus is a multifactorial, polygenic illness marked by an increase in fasting blood glucose due to an absolute or relative insulin insufficiency[1]. A relative or absolute insulin insufficiency is the etiology of diabetes mellitus. Diabetes-induced changes in metabolism have a major impact on bone metabolism, increasing the possibility of fracture approximately six times for type 1 diabetics and two times for type 2 diabetics. Osteoporosis and diabetes are two illnesses that wreak havoc on the senior population, resulting in medical expenses that are two times greater than those of healthy elderly individuals[2]. Real impacts of hyperglycemia, or insulin resistance or insufficiency on the skeleton, and bone marrow environment may produce bone and minerals abnormalities in people with diabetes mellitus. Advanced glycation end products (AGEs), aberrant cytokine, adipokine biosynthesis, their deleterious effects on osteocytes, and altered neuromuscular/skeletal connections are all factors to consider[3].

Vitamin D seems unusual amongst hormones in that it is produced in the skin as a result of sun exposure[4]. There are two different types of vitamin D. Vitamin D2 is made by irradiating the yeast sterol ergosterol with ultraviolet light, and it may be detected within daylight mushrooms. Vitamin D3 is made in the skin and found in oily fish like herring, salmon, and mackerel. A commercially available product of vitamin D3 is 7-dehydrocholesterol, the cholesterol precursor, which is found naturally in the skin, or from lanolin[5].

Leptin of human origin has a sequence of 167 amino acids, the molecular weight of leptin is around (16 kDa) and it has 67% sequence identity among diverse species. Leptin is coded by a specific gene called the obese (ob) gene that is expressed mainly in adipocytes; also small amounts of leptin are secreted by cells in the epithelium, stomach, and placenta. The secreted amount of leptin increases as the body fat increases, it is found that a higher concentration of the specific mRNA is present in adipocytes from obese compared to thin subjects[6].

Even though the physiologic substrate inside the body is unknown, alkaline phosphatase is a concept that represents a set of enzymes that hydrolyze phosphate esters at an alkaline pH. The liver, bone, gut, and (throughout pregnancy, placenta) all contribute to the plasma component of alkaline phosphatase[7]. Osteoblasts, which form the collagen lattice and bony mineral matrix, have a high level of ALP enzyme activity. A rise in alkaline phosphatase action in a blood sample indicates increased osteoblastic action[8].

Following stimulation of the multisystem stressor, hepatic synthesis of proteins switches from constituent proteins (e.g. transferrin and albumin) to acute inflammatory proteins (e.g. C-reactive protein (CRP) and fibrinogen). Therefore, they result in increased plasma levels of CRP, complement proteins, protease inhibitors, and coagulation proteins. All such acute stage proteins are randomly distributed into type I, e.g. α1-acid glycoprotein and haptoglobin that are stimulated by TNF-like cytokines and IL-1[9]. It's very useful for detecting infections in newborns. The normal physiological response against infection, like fever and neutrophil leukocytosis, would not be evident in immunocompromised individuals[10]. CRP serum levels might be able to tell the difference between a fatal and non-fatal acute heart attack. However, this has yet to be shown. Other disorders that predispose to cardiovascular diseases, particularly diabetes, appear to have similar risk increases[11].

Purine metabolism produces serum uric acid, which is processed for further water-soluble allantoin in most other animals[12]. Humans are unable to oxidize uric acid because they lack the uricase enzyme[13]. Uric acid in the blood, on the other hand, has lately been linked to insulin resistance. Uric acid is a chemical that may have a wide range of biological actions, from antioxidant to strong prooxidant. As a result of its biological ambiguity, decreasing uric acid levels are likely to be linked to a higher risk of death as a result of decreased antioxidant activity[14]. Uric acid, the most prominent antioxidant within the plasma, combines directly with nitric oxide (NO) in a quick irreversible process, forming 6-aminouracil and depleting NO, an essential mediator for insulin action, while also improving blood supply and glucose distribution to skeletal muscles. Furthermore, uric acid causes oxidative stress to the human vascular endothelium by activating the renin-angiotensin mechanism[15].

MATERIALS AND METHODS

The current study was approved by the Medical Ethics Committee of Tikrit University / College of Medicine and given the code number (IQ.TUCOM.REC.2019.Bio75). All participants of the study signed an ethical acceptance form based on the World

Medical Association Declaration of Helsinki, amended in 2000, Edinburgh.

The study was conducted by collecting 120 blood samples from 70 patients (35 males and 35 females) with type 2 diabetic individuals and 50 healthy controls (25 males and 25 females). Individuals in the study population ranged (18-50) years old and were hospitalized at Ballad and Salah Aldeen General Hospitals in Salah Aldeen province from August 2019 to February 2020. A five ml blood specimen was taken in a plain tube, then left to clot for 20-30 minutes before centrifuging with a microcentrifuge at 4000 rpm for 5 minutes. Then collected fresh, non-hemolysed serum and stored inside the deep freezer. The serum was separated into two tubes, one of the first series of tests (S. Alkaline Phosphatase and S. Uric Acid) which were performed utilizing spectrophotometer enzymatic procedures. The other second tube included the achieved (Vitamin D and leptin) test, which was performed using the Enzyme-Linked Immunosorbent Assay (ELIZA) sandwich technique (Sunlong). As well as the CRP test that was performed utilizing the I-Chroma device and fluorescent immunoassay technology. The SPSS program version 26 was used to conduct the current findings statistical analysis. A p-value of less than 0.05 was deemed statistically significant.

RESULTS

As indicated in the table and figure (1), the mean ± SD of vitamin D for male diabetes patients and controls was (8.60 ± 3.35) and (20.14 ± 1.83) ng/ml, ranging within (3.3-17.1) and (14.9-24.7) ng/ml, respectively. Whereas the mean ± SD of vitamin D in female diabetes cases and controls, as can be seen in table and figure (1), was (9.62±1.47) and (20.17±1.13) ng/ml, ranging from (3.0-69.6) and (18.0-23.5) ng/ml, respectively. When compared to controls, the results exhibited a highly significant difference (P≤0.01) between female and male diabetes patients.

Table 1. Serum vitamin D levels (ng/ml) in diabetic patients and control

Groups	Number of subject	S.D ± Mean	Range	P. value
Control males	25	1.83 ± 20.14	14.9 – 24.7	P≤0.01
Diabetic males	35	3.35 ± 8.60	3.3 – 17.1	
Control females	25	1.13 ± 20.17	18.0 – 23.5	P≤0.01
Diabetic females	35	1.47 ± 9.62	3.0 – 69.6	

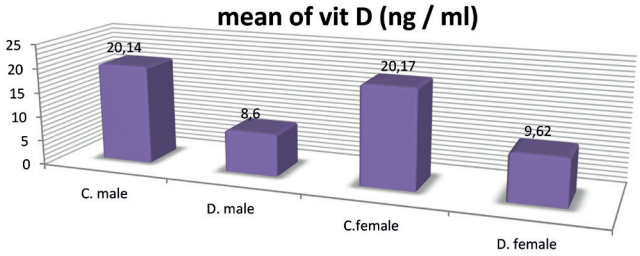

Figure 1. Serum vitamin D levels in diabetic patients and control

As indicated in the table and figure (2), the mean ± SD of leptin levels for male diabetes patients and controls was (14.46±5.62) and (9.55±3.91) ng/ml, ranging within (6.0 – 25.0) and (4.0 – 15.0) ng/ml, respectively. The mean ± SD of leptin for female diabetes cases and controls was (30.78±13.28) and (15.42±8.49) ng/ml, ranging from (10.0 – 55.0) and (5.0 – 30.0) ng/ml, respectively. When compared to controls, the results exhibited a highly significant difference (P≤0.01) between female and male diabetes patients.

Table 2. Serum leptin levels (ng/ml) in diabetic patients and control

Groups	Number of subject	S.D ± Mean	Range	P. value
Control males	25	9.55 ± 3.91	4.0 – 15.0	P≤0.01
Diabetic males	35	14.46 ± 5.62	6.0– 25.0	
Control females	25	15.42 ± 8.49	5.0– 30.0	P≤0.01
Diabetic females	35	30.78 ± 13.28	10.0– 55.0	

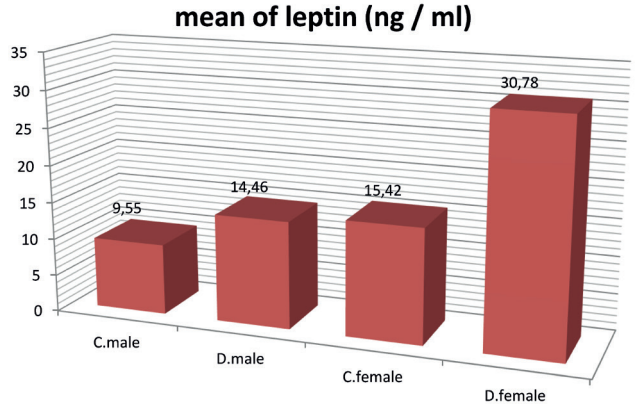

Figure 2. Serum leptin levels in diabetic patients and control

As shown in table and figure (3), the mean ± SD of serum uric acid for male diabetes patients and controls were (6.38±1.21) and (5.26±0.88) mg/dl, ranging from

(4.6-9.5) and (3.9-6.9) mg/dl, respectively. As indicated in table and figure (3), the mean ±SD of serum uric acid for female diabetes patients and controls was (4.57±3.29) and (3.80±1.30) mg/dl, ranging from (2.9-6.5) and (2.9-5.3) mg/dl, respectively. When a comparison with the control group was done, these findings of female and male diabetic patients exhibited a highly significant difference (P≤0.01) difference.

Table 3. Serum uric acid levels (mg/dl) in diabetic patients and control

Groups	Number of subject	S.D ± Mean	Range	P. value
Control males	25	0.88 ± 5.26	3.9 – 6.9	P≤0.01
Diabetic males	35	1.21 ± 6.38	4.6 – 9.5	
Control females	25	1.30 ± 3.80	2.9 – 5.3	P≤0.01
Diabetic females	35	3.29 ± 4.57	2.9 – 6.5	

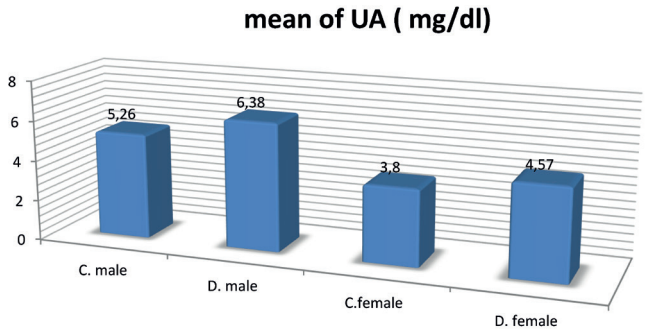

Figure 3. Serum uric acid levels in diabetic patients and control

As shown in the table and figure (4), the mean ± SD of serum ALP for male diabetes patients and controls was (9.40±2.55) and (9.56±0.93) K.A.U/dl ranging from (4.8-13.7) and (8.3-12.0) K.A.U/dl, respectively. The mean±SD of serum ALP for female diabetes patients and controls, as demonstrated by table and Figure 4., was (10.63±4.19) and (9.30±0.78) K.A.U/dl, ranging from (4.5-21.9) and (8.0-11.0) K.A.U/dl respectively. When female and male diabetic patients were compared to the control group, these findings exhibited no significant differences (P≥0.05).

Table 4. Serum alkaline phosphatase (K.A.U/dl) levels in diabetic patients and control

Groups	Number of subject	S.D ± Mean	Range	P. value
Control males	25	0.93 ± 9.56	8.3 – 12.0	P≥0.05
Diabetic males	35	2.55 ± 9.40	4.8 – 13.7	
Control females	25	0.78 ± 9.30	8.0 – 11.0	P≥0.05
Diabetic females	35	4.19 ± 10.63	4.5 – 21.9	

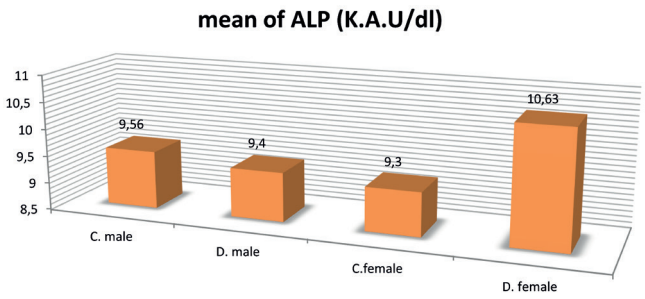

Figure 4. Serum alkaline phosphatase levels in diabetic patients and control

As indicated in table and figure (5), the mean ± SD of C-reactive protein in male diabetes patients and controls was (17.69±13.15) and (3.39±0.83) mg/dl, ranging from (8.76–70.0) and (2.2-5.0) mg/dl, respectively. As indicated in table and figure (5), the mean ±SD of C-reactive protein among female diabetes patients and controls was (16.46±9.38) and (4.04±1.31) mg/dl, ranging from (7.24-60.8) and (2.5-6.4) mg/dl, respectively. When a comparison with the control group was done, these findings of female and male diabetic patients exhibited a highly significant difference (P≤0.01) difference.

Table 5. C-Reactive protein levels (mg/dl) in diabetic patients and control

Groups	Number of subject	S.D ± Mean	Range	P. value
Control males	25	0.83 ± 3.39	2.2 – 5.0	P≤0.01
Diabetic males	35	13.1 ± 17.69	8.76 – 70.0	
Control females	25	1.31 ± 4.04	2.5 – 6.4	P≤0.01
Diabetic females	35	9.38 ± 16.46	7.24 – 60.8	

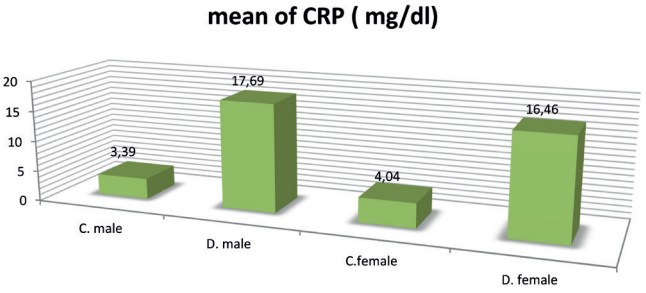

Figure 5. C-Reactive protein levels in diabetic patients and control.

DISCUSSION

Vitamin D levels in diabetes patients, both male and female, were found to be considerably lower than in the control group in this investigation. These findings were in agreement with those of Esteghamati A[16], Fatih S[17], Rija F[18], and Al-Jebawi AF[19], although no contrary evidence had been discovered. The combination to Vitamin D receptors (VDRs) that induce expression of the insulin receptors onto target tissues has been proven in several investigations to enhance insulin sensitivity. Vitamin D3 conversion towards its derivative 1,25 vitamin D3 is a complicated process that includes several hormones. 1,25 vitamin D3 is identified to be an efficacious regulator of the cell differentiation and proliferation by its receptor, that is existent in insulin-forming beta cells. This could indicate that hypovitaminosis D3 is linked to T2DM's long-term, grievously abnormal (carbohydrate) metabolism and muscle insulin resistance. Vitamin D may also enhance insulin release by regulating intracytoplasmic calcium concentration in beta cells and stimulating the exocytosis process. Vitamin D, on the other hand, has an apoptotic and anti-insulinogenic action on neoplastic β-cell lines. In fact, calcitriol slows growth, induces apoptosis, and reduces cell viability, insulin content, gene expression, and secretion in murine insulinoma β-cells, according to several cellular researches[20].

Leptin levels were increased in male and female diabetic patients when compared with control. These results were agreed with the results found by Hussein SZ[21], Peng X et al[22], Huang J et al[23], & Hussein SZ[24] but disagreed with that of Onyemelukwe OU et al[25], Chen CC et al[26].

Serum leptin level in male diabetic patients was found higher than in control because of decreasing testosterone level in male diabetic patients rather than control when male was associated negatively with serum leptin independently of body fatness. Serum leptin level in female was found higher than in male and this is probably owing to adipose tissue in female being higher than that in male, the existence of negative correlation between leptin and testosterone levels and the stimulation of mRNA production by 17 β-estradiol, which was one of the female sexuality hormones[22].

Male and female diabetes individuals had greater serum uric acid level than control, while male diabetic individuals had higher serum uric acid level than female diabetic individuals. These findings matched those of Kodama S[27] and Zong J[28] but did not match those of Taniguchi Y[29], Abdalla MA et al[30], and Hussin SJ et al [31]. Uric acid is a significant water-soluble endogenous antioxidant in the human body. Enhanced oxidative stress is linked to diabetes and associated vascular consequences, according to a growing body of research. As a result, higher plasma uric acid levels might indicate that the body is attempting to defend itself from the damage caused by free radicals by boosting endogenous antioxidant compounds, such as uric acid. Throughout the oxidative stress, uric acid inhibits endothelial enzymes from being oxidized and retains the endothelium's capacity to mediate vascular dilation. The metabolic syndrome was found to have a role in the relationship between diabetes and hyperuricemia[28].

When compared to controls, ALP decreased somewhat in diabetes males but elevated in diabetic females, indicating that ALP elevated in diabetic females rather than diabetic males. These findings were in agreement with those of Zhang Y[32], Sinambela TH[33], Zhao C[34] and Rija FF[35], but not with those of Hussein SZ[36] and Nasir AH[37]. Insulin increases bone matrix production when combined with insulin-like growth factor. Insulin's influence on the differentiation function of osteoblasts causes it to have a stimulatory effect on bone matrix. Normal bone mineralization requires insulin as well. Hyperglycemia accelerates alkaline phosphatase interaction and expression while decreasing osteocalcin activity. Osteoblasts regulate mineralization through controlling the transmission of phosphate and calcium ions through their cell membrane. Those cells also possess alkaline phosphatase, which converts inorganic phosphates to phosphate ions[38].

Female and male diabetes patients had considerably more C-reactive protein than controls, and male diabetic patients had significantly higher C-reactive protein than female diabetic patients. These findings were in agreement with Amanullah S[39], Ali S[40] and Hussein SZ[41]; however there were no accessible findings that contradicted these findings. The major inflammatory component generated by liver cells during an inflammation or acute infection is C- reactive protein (CRP). The CRP levels that are elevated are linked to the development of insulin resistance. CRP, a sensitive physiological measure of subclinical systemic inflammation, is linked to hyperglycemia, insulin resistance, and observable type 2 diabetes mellitus, regarding to experimental findings and some cross-sectional data. Insulin resistance and decreased insulin secretion are

the most commonly associated abnormality in type 2 diabetics; however the exact cause of these metabolic abnormalities is unknown. Inflammation is assumed to have a crucial role in the development of type 2 diabetes, connecting diabetes with a variety of typically co-existing conditions thought to be caused by inflammatory mechanisms[42-45].

CONCLUSIONS

Patients with type 2 diabetes have severe vitamin D insufficiency and increased leptin levels, which leads to a rise in C-reactive protein and complicates bone metabolic illness due to an increase in serum uric acid and a change in ALP.

Compliance with ethics requirements: The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

References

1. Artasensi A, Pedretti A, Vistoli G, Fumagalli L. Type 2 diabetes mellitus: A review of multi-target drugs. Molecules. 2020 Apr;25(8):1987.
2. Kasperk C, Georgescu C, Nawroth P. Diabetes mellitus and bone metabolism. Exper & Cli Endocri & Diab. 2017;125(4).
3. Liu XX, Jiang L, Liu Q, Zhang J, Niu W, Liu J, et al. Low bone turnover markers in young and middle-aged male patients with Type 2 diabetes mellitus. J Diab Res. 2020:1-8.
4. Abdalla MA, Nayaf MS, Hussein SZ. Evaluation of vitamin D in melasma patients. Rev Romana Med Lab. 2019;27(2):219-221.
5. Nayaf MS, Ahmed AA, Abdalla MA. Alopecia areata and serum vitamin D in Iraqi patients: A case-control study. Prensa Med Argent. 2020;106(3):287.
6. Hussein SZ, Al-Samarrai AH. Leptin level in gestational diabetes mellitus. Tikrit Med J. 2012;18(2):169-174.
7. Bhattarai T, Bhattacharya K, Chaudhuri P, Sengupta P. Correlation of common biochemical markers for bone turnover, serum calcium, and alkaline phosphatase in post-menopausal women. Malaysian J Med Sci. 2014;21(1):58.
8. Mohammad SA, Abdalla MA. The frequency of ossification of the sacrococcygeal joint in Iraqis. Tikrit Med J. 2007;13(1):95-98.
9. Eckersall PD. Proteins, proteomics, and the dysproteinemias. Clin Biochem Domesc Anim. 2008;6:117-156.
10. Shihan JT, Al-Janabi MSA, Abdalla MA. Impact of aqueous extract of ginger and chamomile plant and some antihistamines in the treatment of drug-allergy induced in white rats, a physiological and immunological study. HIV Nursing. 2022;22(2):3449-3454.
11. Dehghan A, Kardys I, de Maat MP. Genetic variation, C-reactive protein levels, and incidence of diabetes. Diabetes. 2007;56(3):872-878.
12. Guerreiro S, Ponceau A, Toulorge D, Martin E, Alvarez-Fischer D, Hirsch EC, et al. Protection of midbrain dopaminergic neurons by the end-product of purine metabolism uric acid: potentiation by low level depolarization. J Neurochemistry. 2009;109(4):1118-1128.
13. Hussein SZ, Abdalla MA. Serum levels of alpha-melanocyte stimulating hormone, vitamin D, calcium, phosphorus and magnesium in COVID-19 patients. Ukr Biochem J. 2021;93(6):64-69.
14. Lamacchia O, Fontana A, Pacilli A, Copetti M, Fariello S, Garofolo M, et al. On the non-linear association between serum uric acid levels and allcause mortality rate in patients with Type 2 diabetes mellitus. Atherosclerosis. 2017;1:260.
15. Vaishnav D, Holkar S, Hivre M. Uric acid in hypertension: A marker of oxidative stress. Inter J Health Sci & Res. 2014;4(12):206-210.
16. Esteghamati A, Aryan Z, Esteghamati A, Nakhjavani M. Vitamin D deficiency is associated with insulin resistance in nondiabetics and reduced insulin production in type 2 diabetics. Horm Metab Res. 2015;47:273-279.
17. Fatih Ş. 25 hydroxy vitamin D3 levels in type 2 diabetic patients. Eur J Med Sci.2015;2(2):48-50.
18. Rija FF, Hussein SZ, Abdalla MA. Osteoprotegerin, sclerostin, and osteocalcin serum levels in thyroid disorder patients. Ukr Biochem J. 2021;93(5):117-121.
19. Al-Jebawi AF, Youssef Agha AH, Al Suwaidi. Attenuated PTH responsiveness to vitamin D deficiency among patients with Type 2 diabetes and chronic hyperglycemia. Diabetes Res & clin Prac. 2017;128:119-126.
20. Altieri B, Grant WB, Della Casa S, Orio F, Pontecorvi A, Colao A, et al. Vitamin D and pancreas: the role of sunshine vitamin in the pathogenesis of diabetes mellitus and pancreatic cancer. Cri Rev food Sci & Nutr. 2016;2:3472-2488.
21. Hussein SZ, Al-Samarrai AM. Hormonal disturbance in normal and diabetic postmenopausal women. Med J Tikrit Uni. 2017;22(1):10-15.
22. Peng X, Huang J, Zou H, Peng B, Xia S, Dong K, et al. Roles of plasma leptin and resistin in novel subgroups of Type 2 diabetes driven by cluster analysis. Lipids Health Dis. 2022;21(1):7.
23. Huang J, Peng X, Dong K, Tao J, Yang Y. The association between insulin resistance, leptin, and resistin and diabetic nephropathy in Type 2 diabetes mellitus patients with different body mass indexes. Diabetes Metab Syndr Obes. 2021;14:2357-2365

24. Hussein SZ, Abdalla MA. Levels of leptin, adiponectin, and insulin in COVID-19 patients. Medicina Moderna. 2022;29(1):89-92.
25. Onyemelukwe OU, Ogoina D, Onyemelukwe GC. Leptin concentrations in type 2 diabetes and non-diabetes Nigerian-Africans. Am J Cardiovasc Dis. 2020;10(4):444-454.
26. Chen CC, Li TC, Chang CT, Liu CS, Lin WY, Wu MT, et al. Lower serum leptin concentration in female subjects with type 2 diabetes mellitus. Mid Taiwan J Med. 2003;8:8-12.
27. Kodama S, Saito K, Yachi Y, Asumi M, Sugawara A, Totsuka K, et al. Association between serum uric acid and development of type 2 diabetes. Diabetes Care. 2009;32(9):1737-1742.
28. Zong J, Sun Y, Zhang Y, Yuan J, Wang X, Zhang R, et al. Correlation between serum uric acid level and central body fat distribution in patients with Type 2 diabetes. Diabetes Metab Syndr Obes. 2020;13:2521-2531.
29. Taniguchi Y, Hayashi T, Tsumura K, Endo G, Fujii S, Okada K. Serum uric acid and the risk for hypertension and Type 2 diabetes in Japanese men: The Osaka Health Survey. J Hypertension. 2001;19(7):1209-1215.
30. Abdalla MA, Nayaf MS, Hussein SZ. Correlation between serum α-MSH and vitamin D levels in vitiligo patients. Iran J Dermatology. 2020;23(4):163-167.
31. Hussin SJ, Hilal NN, Hussein SZ. The changes of serum irisin levels in postmenopausal breast cancer women. Int J Health Sci. 2022;6(S6):7007-7015.
32. Zhang Y, Zhou C, Li J. Serum alkaline phosphatase levels and the risk of new-onset diabetes in hypertensive adults. Cardiovasc Diabetol. 2020;19:186.
33. Sinambela TH, Pramudianti MID, Ariningrum D. Correlation between plasma osteopontin and alkaline phosphatase in Type 2 diabetes mellitus patients. Indonesian J Clin Pathol & Med Lab. 2020 March;26(2):168-174.
34. Zhao C, Liu G, Zhang Y, Xu G, Yi1X, Liang J, et al. Association between serum levels of bone turnover markers and bone mineral density in men and women with type 2 diabetes mellitus. J Clin Lab Anal. 2020;34:e23112.
35. Rija FF, Almahdawi ZMM, Hussein SZ. Correlation between calcium sensing receptor with other calcium regulators in osteoporosis and osteomalacia patients. Plant Archives. 2019;19(1):1199-1207.
36. Hussein SZ. Evaluation of thyroid hormones and ferritin level in patients with β-thalassemia. Med Pharm Rep. 2022;95(2):152-157.
37. Nasir AH, Linda BE, Sara LB. Clinical study of serum calcium, phosphorus, and alkaline phosphates level in Type II diabetes mellitus among Sudanese population in Khartoum state. NMJ June.2013;3(10):1858-1927.
38. Rija FF, Hussein SZ, Abdalla MA. Physiological and immunological disturbance in rheumatoid arthritis patients. Baghdad Sci J. 2021;18(2):247-252.
39. Amanullah S, Jarari A and Govindan M. Association of hs-CRP with diabetic and non-diabetic individuals. Jordan J Bio Sci. 2010;3(1):7-12.
40. Ali SJ, Hussein SZ, Hussain WN. Study of the correlation between serum C-reactive protein and bone biochemical markers in Type 2 diabetic individuals. Tikrit J Pure Sci. 2014; 19(5):58-64.
41. Hussein SZ. The relationship of some biochemical markers with inflammatory prognosis in COVID-19 patients. Medicina Moderna. 2022;29(3):241-246.
42. Hansen D, Dendale P, Beelen M, Jonkers RA, Mullens A, Corluy L, et al. Plasma adipokine and inflammatory marker concentrations are altered in obese, as opposed to non-obese, type 2 diabetes patients. Euro J Appl Physio. 2010;109(3):397-404.
43. AL-Robaiee SWS, Hussein SZ. The relation between parathyroid hormone with some bone biochemical markers in Type II diabetes mellitus. Medicina Moderna. 2022;29(1):59-65.
44. Hammad MJ, Hassan ZL, Hussein SZ. The relation between some vitamins and homocysteine levels with pre and postmenopausal osteoporosis women. HIV Nursing. 2022;22(2):2439-2442.
45. Hammad MJ, Hassan ZL, Hussein SZ. Evaluation of some adipokines levels in pre and postmenopausal osteoporosis women. HIV Nursing. 2022;22(2):2345-2348.