RESEARCH ARTICLE

Chromium status and insulin resistance as the risk factors of ischemic heart disease in Type 2 Diabetes Mellitus patients

Ahmed Hashim Abbass Al-Janabi[1], Mohammed Alaa Abdulzahra[2], Salim Shamkhi Jaafar[2], Thaer Shafi Hussein[3], Yasir Haider Al-Mawlah[2, *]

[1]GIT center, Karbala Health Directorates, Karbala, Iraq. [2]DNA research Center, University of Babylon, Babylon, Iraq. [3]Department of Pharmacy, Al-Amal College for Medical Specialized sciences, Karbala, Iraq.

Received: June 7, 2023; **accepted:** July 7 , 2023.

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder characterized by the presence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids, and proteins. One of important complications of T2DM is ischemic heart disease (IHD) which is an important risk factor for the development of systolic and diastolic dysfunction of the heart and is intricately linked to their pathophysiology. Metals are essential cofactors that play a crucial role in heart function at the cell and tissue levels such as chromium, zinc, cobalt, selenium, and nickel. Chromium and its compounds are absorbed in the human body through the exposure to oral, dermal, and inhalation routes. However, the information regarding the role of metals in the pericardial fluid and its ionome in IHD is limited. This study aimed to investigate the associations between various biomarkers and chromium ion levels in both T2DM patients with and without IHD. In addition, the correlations between various biomarkers were determined. 50 T2DM patients with IHD (28 male and 22 female) aged from 45 to 76 years old admitted to Al-Hussein Medical City, Kerbala Health Directorates, Kerbala, Iraq between November 2020 and August 2021 were involved in this study, while another 50 T2DM patients without IHD (24 male and 26 female) aged from 49 to 82 years old were included in this study as control. The results showed that, when the chromium level was less than 4.25 part per billion (ppb), the individual was classified as having cardiovascular disease. The element chromium had a potential association with IHD and had been designated as a prediction marker. IHD is associated with serious health problems such as atherosclerosis, myocardial ischemia, health-related behaviors, and other biological risk factors. The results found that the Analytical Ultracentrifugation (AUC) value was 0.903, which was more than 0.5 indicating that the chromium cases could be predicted with a high degree of accuracy (95.76 %), also indicating a better effect. The results of this study were benefit to understand the impact of chromium status and insulin resistance on the development of IHD in individuals with T2DM, and had significant implications for patient management and public health.

Keywords: chromium ion; diabetes mellitus; ischemic heart diseases; insulin resistance.

[*]**Corresponding author:** Yasir Haider Al-Mawlah, DNA research Center, University of Babylon, Babylon 51001, Iraq. Phone: +964 770 571 3626. Email: Yasser.almawla@uobabylon.edu.iq.

Introduction

Diabetes mellitus, commonly known as diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period. The most important symptoms are polyuria, weight loss, and constant thirst [1]. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, hyperosmolar

hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, damage to the nerves, damage to the eyes, and cognitive impairment [2]. It is well established that ischemic heart diseases (IHD) are the global health issue and the major cause of mortality and morbidity worldwide [3]. This condition is characterized by an inadequate supply of oxygen inside the cardiac muscles because of an imbalance between oxygen supply and demand, as well as heart disease caused by coronary artery stenosis. IHD and ischemic stroke are both frequent conditions with comparable pathogenesis based on arteriosclerosis which affects the patients all over and puts them at risk for both acute coronary syndrome (ACS) and acute stroke. In both circumstances, there is an abrupt shift in circulation, resulting in reduced blood flow to a portion of the heart or brain. Stroke has been compared as a "heart attack" in the brain on occasion [4, 5].

Metals are essential cofactors that play a crucial role in heart function at the cell and tissue levels such as chromium, zinc, cobalt, selenium, manganese, and nickel. Information regarding the role of metals in the pericardial fluid and its ionome in IHD is limited. Micronutrients are essential cofactors needed only in small amounts for energy transfer in cells and thus play a crucial role in heart function at the cell and tissue levels [6]. These metals play a role as regulators of oxidative stress, antioxidants, and regulators of inflammatory response and immune cell activity [7]. There is emerging evidence suggesting an important role of trace elements like chromium, zinc, cobalt, selenium, manganese, and nickel in the heart and that their homeostasis imbalance may lead to an increase in the risk of cardiac remodeling in heart failure. Studies showed that these micronutrients were intricately linked to IHD [8]. Chromium (Cr) is the most abundant mineral in Earth's crust. Cr has an atomic number of 24 in the periodic table and a relative atomic mass of 51.996. It occurs in almost all oxidation states ranging from -2 to +6. However, Cr is mostly stable in trivalent and hexavalent form in environment. Cr presented in zero oxidation state is biologically inert and is not naturally present in Earth's crust, while Cr (III) and Cr (VI) are originated from industries. The available forms of chromium are halides, oxides, and sulphides, which is the +2 oxidation state of chromium and is unstable and can be easily oxidized to +3 forms in the presence of air [9]. Chromium and its compounds are absorbed in the human body through the exposure to oral, dermal, and inhalation routes. Cr (III) is less absorbed than Cr (VI), which leads to a difference in their transport methods to cells. Cr (VI) enters the cell *via* a non-specific anion channel by facilitated diffusion, while Cr (III) enters the cell by passive diffusion or phagocytosis. Human liver, kidney, spleen, and bone have more concentration of Cr than other organs [10]. Cr (VI) can easily penetrate the RBC. Because of its bioavailability, Cr (VI) enters into RBC and is converted into Cr (III) which binds to the cellular components and then is unable to leave RBC. The structure of cells somewhat resembles the structure of RBC. Therefore, Cr (VI) can be easily uptake by other cells. Absorption of Cr depends on some factors including particle size, oxidation state, and solubility, but majorly on the interaction with biomolecules in lungs. The main reduction of Cr (VI) to Cr (III) takes place in lung tissues [10, 11]. This study investigated the association of serological chromium elements with type 2 diabetes mellitus (T2DM) patients with and without IHD, and further, studied the correlations of Cr with lipid profile, fasting blood sugar, insulin, insulin resistance, and glycated hemoglobin A1c percentage (HbA1c %). The results of this study might be important implications for patient care and public health because it would help to understand how chromium status and insulin resistance affected the onset of IHD in people with T2DM.

Materials and Methods

Patient information and blood sample collection

Two groups of T2DM patients including 50 patients with IHD (28 males and 22 females, aged

from 45 to 76 years old) and 50 patients without IHD (24 males and 26 females, aged from 49 to 82 years old) were included in this study. The study was conducted between November 2020 to August 2021 and all procedures were approved by the Research Ethical Committee of the University of Babylon, Babylon, Iraq. 5 mL of blood samples were collected by vein puncture after obtained the written informed consent from each participant in the Kerbala Heart Center, Al-Hassan Center for Endocrinology and Diabetes, Al-Hussein Teaching Hospital, and Al-Hussein Medical City (Kerbala Health Directorates, Kerbala, Iraq). The biomarkers identification and molecular studies were performed in the laboratories of Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala and Al-Hussein Teaching Hospital laboratories (Kerbala, Iraq).

Assays for blood chromium, glucose, HbA1c, and insulin detection

Each blood sample was divided into three portions with the first part for serum separation to determine chromium ion and glucose levels, the second part in EDTA tube for the determination of glycated hemoglobin (HbA1c), and the third part for the detection of serum insulin concentration.

The chromium ion levels were determined by using Shimadzu 6300 atomic absorption spectrophotometry (Shimadzu, Kyoto, Japan) with standard solution of chromium ion at 1,000 mg/L). Two main gas mixtures were used for the source flames including air-acetylene and nitrous oxide-acetylene. The air-acetylene was used for elements that were not prone to refractory conditions. When an element presented as an oxide and wasn't transformed into a gaseous state in the flame, the refractory circumstances prevailed [12]. Five concentrations of working standard chromium solutions were prepared from stock solution of 1,000 part per million (ppm) as 2.5, 5, 7.5, 10, and 12.5 part per billion (ppb). The measurements were taken at the wavelength of 357.9 nm by using lamp current low of 10 mA and lamp mode of BGC-D2 with slit width tube of 0.7 nm.

The blood HbA1c levels were determined by using COBAS HbA1c kit and COBAS INTEGRA© 400 plus analyzer (Roche Diagnostics, Basel, Switzerland) following the manufacturer's instructions. The normal level of HbA1c was set as less than 7% with the risk level equal to or more than 7% [13].

The serum insulin concentrations were detected through a one-step immunoassay by using the kit from Abbott Laboratories, Abbott Park, IL, USA following the manufacturer's instructions.

Calculation of insulin resistance

The insulin resistance measurement or homeostatic model assessment of insulin resistance (HOMA-IR) was calculated by using the following equation which illustrated both the current presence and extent of any insulin resistance. It was a terrific way to reveal the dynamic between baseline (fasting) blood sugar and the responsive hormone insulin. However, the HOMA-IR equation was an approximating calculation for insulin resistance [13].

HOMA-IR = Fasting insulin (mlU/L) x Fasting glucose (mg/dL) / 405

Statistical analysis

SPSS version 23.0 (IBM, Armonk, New York, USA) was employed in this study for statistical analysis. The data from two groups were expressed as mean ± SD. Student t-test was applied to compare the difference between two groups. The whole number of incidences of the tested allele in the population was divided by the whole number of alleles to compute allele frequencies. The odds ratio (OR), 95% confidence intervals, and *P* values of genotype distributions and allele frequencies were calculated by using the Hardy-Weinberg equilibrium assumption and a Chi-square test. The $P < 0.05$ was set as significant difference.

Results and discussion

The patients' information involved in this study was listed in Table 1. Because of the under-recognition of cardiac disease and the variations in clinical presentation between male and female, less aggressive treatment options were applied to female patients and women were also neglected in clinical trials. Therefore, it is necessary to bring in more attentions to increase the self-awareness and identify the cardiovascular risk factors for female patients, which will lead to better cardiovascular event prevention [14].

Table 1. Patients' information.

Gender	T2DM patient		Total
	Without IHD	With IHD	
Female	22 (44.0%)	24 (48.0%)	46 (46.0%)
Male	28 (56.0%)	26 (52.0%)	54 (54.0%)

The HbA1c levels in both T2DM with/without IHD

The mean ± SD of HbA1c% in T2DM patients with IHD was 9.674 ± 1.72% which was slightly non-significantly higher than that in T2DM patients without IHD (9.64 ± 2.087%) (P > 0,05). The results were disagreement with the previous study which found that HbA1c was associated with cardiovascular disease (CVD) such as carotid and coronary artery atherosclerosis, IHD, ischemic stroke, and hypertension among other things, and was related to dyslipidemia, hyperhomocysteinemia, hypertension, the increase of C-reactive protein level, oxidative stress, and blood viscosity, which all were associated with the development of cardiovascular illnesses [15].

The insulin levels in both T2DM with/without IHD

The insulin levels in both groups were 6.86 ± 4.31 µU/mL and 6.03 ± 5.234 µU/mL for T2DM with IHD and without IHD, respectively. The results demonstrated a non-significantly higher insulin level in T2DM with IHD group than that in T2DM without IHD group (P > 0.05) (Table 2). Cardiovascular illnesses are the leading cause of death worldwide [16]. The T2DM is one of factor to cause death because it is so common and doubles the risk of heart disease. Increased glucose and insulin concentrations, as a result, had been proven to be proatherogenic causes [17], whereas other study showed that cardiovascular diseases might be a consequence of insulin resistance rather than being caused by toxic effects of high insulin or glucose concentrations [18].

Determination of HOMA-IR levels

The level of HOMA-IR found in T2DM patients with IHD was 3.351 ± 2.38, while it was 2.65 ± 2.41 in T2DM without IHD patients (P > 0.05) (Table 2). The assessment of HOMA-IR has been widely used to validate the diagnosis of insulin resistance, which includes both glucose and insulin concentrations. Insulin resistance could increase the risk of atherosclerosis through a variety of pathways [19] and has been linked to coronary artery disease.

The fasting glucose levels

Table 2 also showed the results concerning the fasting blood glucose (FBG) levels in sera of T2DM with/without IHD. The mean ± SD of FBG levels were determined in both groups as 198.9 ± 42.283 mg/dL in T2DM with IHD and 185.5 ± 56.77 mg/dL T2DM without IHD, which was non-significantly higher in T2DM with IHD group than that in T2DM without IHD (P > 0.05). The impact of hyperglycemia on coronary heart disease (CHD), stroke, and other cardiovascular diseases (CVDs) had been widely studied [20-22]. In people with hyperglycemia, two-hour plasma glucose (2hPG) was a better predictor of coronary heart disease (CHD) and ischemic stroke (IS) than fasting plasma glucose (FPG). However, nothing is known regarding their impacts in the normoglycemic range. Insulin resistance and beta cell dysfunction were already evident in people with increased normal FPG [23, 24].

Table 2. The levels of HbA1c, insulin, HOMA-IR, and blood glucose concentration in T2DM with/without IHD.

		Number	Mean ± SD	*P* value
HbA1c%	**Without IHD**	50	9.64 ± 2.087	0.921
	With IHD	50	9.674 ± 1.72	
	Total	100	9.66 ± 1.9	
Insulin, µU/mL	**Without IHD**	50	6.03 ± 5.234	0.392
	With IHD	50	6.86 ± 4.31	
	Total	100	6.45 ± 4.79	
HOMA-IR	**Without IHD**	50	2.65 ± 2.41	0.145
	With IHD	50	3.351 ± 2.38	
	Total	100	3.0 ± 2.41	
FBG, mg/dL	**Without IHD**	50	185.5 ± 56.77	0.184
	With IHD	50	198.9 ± 42.283	
	Total	100	192.2 ± 50.256	

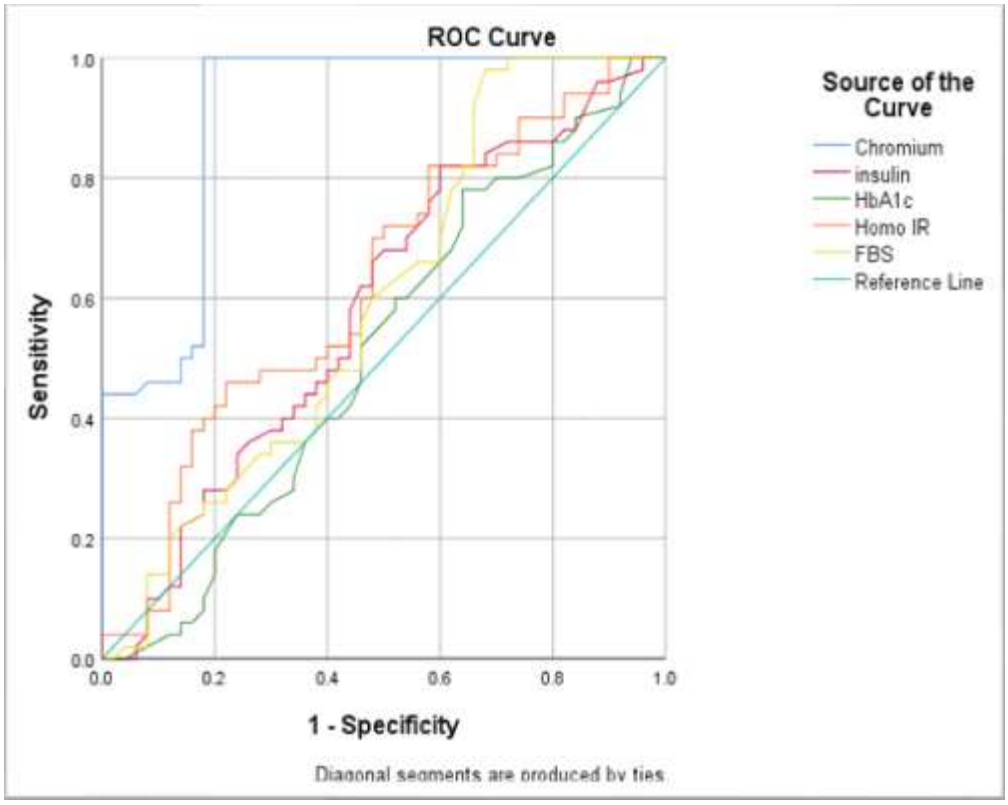

Figure 1. ROC curve analysis. The true positive rate (TPR) and false negative rate (FPR) were plotted on a two-dimensional graph for prediction of some blood parameters.

The receiver operating characteristic (ROC) curve analysis

The ROC curve analysis is used by medical experts to investigate diagnosis performance. The area under the curve (AUC) was used to measure the ROC plots that were applied to evaluate the performance of each categorized blood parameter value. The curve's value was between 0 and 1, which indicated the model's overall reliability. When the value of curve was 1.0, it

Table 3. Area under the curve (AUC) to analyze of some blood parameters in the T2DM patients with IHD.

Tests	AUC	Standard Error[a]	Asymptotic Significant[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Chromium (ppb)	0.903	0.032	0.000	0.841	0.966
Insulin (µU/mL)	0.584	0.057	0.150	0.471	0.696
HbA1c	0.513	0.059	0.828	0.398	0.627
HOMA-IR	0.622	0.056	0.035	0.512	0.733
FBS (mg/dL)	0.596	0.058	0.099	0.483	0.709

Notes: [a]under the nonparametric assumption. [b]null hypothesis: true area = 0.5.

indicated great sensitivity and specificity [25]. In this study, the AUC was utilized to assess the accuracy of each parameter category. An AUC of 1.0 suggested that the following test findings including chromium status in the data set could be predicted without error. An AUC of 0.50, on the other hand, indicated a 50% chance of accurately predicting insulin, HbA1c, HOMA-IR, and FBG categories. As shown in Figure 1, the stronger classifier should be set near the left corner of the ROC plot's height.

Determination of chromium levels

When the chromium level was less than 4.25 ppb, the individual was classified as having cardiovascular disease. The element chromium had a potential association with IHD and had been designated as a prediction marker [26–28]. IHD is associated with serious health problems including atherosclerosis, myocardial ischemia, health-related behaviors, and other biological risk factors. The result of this study found that the AUC value was 0.903, which was more than 0.5, indicating that the chromium levels could be predicted with a high degree of accuracy (95.76 %) (Table 3).

Based on numerous research and clinical trials, Cr has proved the key in the prevention or alternative therapy in treating diabetes or as vital elements in lowering hyperlipidemia. Many previous studies demonstrated in favor of positive effects of Cr compounds on diabetes mellitus (DM) and lipid profile, while very few reports showed no effect. However, none of the trials showed a negative effect of Cr on DM. Numerous literatures in both animal and human experiments and trials demonstrated and supported the hypothesis that Cr was an essential micronutrient involved in insulin metabolism as the results showing the Cr deficiency on T2DM patients. It suggested that deficiency of the above trace elements played a crucial role in developing DM. Cr has the effect of preventing or delaying the steady progress of pre-diabetes to diabetes. Multivitamin intake which contains Cr pollinate or brewer's yeast would reduce or delay diabetic onset. By prohibiting or postponing diabetes invasion, the risk of other DM comorbid diseases and conditions could be reduced.

References

1. Nordestgaard BG, Palmer TM, Benn M, Zacho J, Tybjaerg-Hansen A, Davey Smith G, *et al*. 2012. The effect of elevated body mass index on ischemic heart disease risk: causal estimates from a Mendelian randomisation approach. PLoS Med. 9(5):e1001212.
2. Burchfiel CM, David Curb J, Rodriguez BL, Abbott RD, Chiu D, Yano K. 1994. Glucose intolerance and 22-year stroke incidence. The Honolulu Heart Program. Stroke. 25(5):951-957.
3. Shelby MD. 2008. NTP-CERHR monograph on the potential human reproductive and developmental effects of bisphenol A. NTP CERHR MON. 2008(22): v, vii-ix, 1-64 passim.
4. De Silva DA, Fung PW, Kyaw TM, Chen CLH, Hui MC, Meng CW. 2008. Concomitant coronary artery disease among Asian ischaemic stroke patients. Ann Acad Med Singapore. 37(7):573-575.
5. Choi D, Hwang KC, Lee KY, Kim YH. 2009. Ischemic heart diseases: current treatments and future. J Control Release. 140(3):194-202.

6. Doi Y, Ninomiya T, Hata J, Fukuhara M, Yonemoto K, Iwase M, *et al*. 2010. Impact of glucose tolerance status on development of ischemic stroke and coronary heart disease in a general Japanese population: the Hisayama study. Stroke. 41(2):203-209.
7. Muti'ah M, Siahaan J, Loka IN, Irawan J. 2022. The efficiency of heavy metal analysis method in marine fish samples by atomic absorption spectrophotometry. Jurnal Penelitian Pendidikan IPA, 8(2):963-968.
8. Abebe W, Liu JY, Wimborne H, Mozaffari MS. 2010. Effects of chromium picolinate on vascular reactivity and cardiac ischemia-reperfusion injury in spontaneously hypertensive rats. Pharmacol Rep. 62(4):674-682.
9. Hyvärinen M, Qiao Q, Tuomilehto J, Laatikainen T, Heine RJ, Stehouwer CD, *et al*. 2009. Hyperglycemia and stroke mortality: comparison between fasting and 2-h glucose criteria. Diabetes care. 32(2):348-354.
10. Lau LH, Lew J, Borschmann K, Thijs V, Ekinci EI. 2019. Prevalence of diabetes and its effects on stroke outcomes: a meta-analysis and literature review. J Diabetes Investig. 10(3):780-792.
11. Michaels AD, Kennard ED, Kelsey SE, Holubkov R, Soran O, Spence S, *et al*. 2001. Does higher diastolic augmentation predict clinical benefit from enhanced external counterpulsation?: data from the International EECP Patient Registry (IEPR). Clin Cardiol. 24(6):453-458.
12. McPherson R. 2015. Obesity and ischemic heart disease: defining the link. Circ Res. 116(4):570-571.
13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. 1985. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 28:412-419.
14. Moradi F, Maleki V, Saleh-Ghadimi S, Kooshki F, Pourghassem Gargari B. 2019. Potential roles of chromium on inflammatory biomarkers in diabetes: A Systematic. Clin Exp Pharmacol Physiol. 46(11):975-983.
15. McKeag NA, McKinley MC, Woodside JV, Harbinson MT, McKeown PP. 2012. The role of micronutrients in heart failure. J Acad Nutr Diet. 112(6):870-886.
16. Nordestgaard BG, Palmer TM, Benn M, Zacho J, Tybjaerg-Hansen A, Davey Smith G, *et al*. 2012. The effect of elevated body mass index on ischemic heart disease risk: causal estimates from a Mendelian randomisation approach. PLoS Med. 9(5):e1001212.
17. Nowbar AN, Gitto M, Howard JP, Francis DP, Al-Lamee R. 2019. Mortality from ischemic heart disease: Analysis of data from the World Health Organization and coronary artery disease risk factors From NCD Risk Factor Collaboration. Circulation: cardiovascular quality and outcomes. 12(6):e005375.
18. Brown E, Wilding JPH, Alam U, Barber TM, Karalliedde J, Cuthbertson DJ. 2021. The expanding role of SGLT2 inhibitors beyond glucose-lowering to cardiorenal protection. Ann Med. 53(1):2072-2089.
19. Chatterjee S. 2015. Chromium toxicity and its health hazards. Int J Adv Res. 3(7):167-172.
20. Pavesi T, Moreira JC. 2020. Mechanisms and individuality in chromium toxicity in humans. J Appl Toxicol. 40(9):1183-1197.
21. Dianyi Yu. 2008. Agency for Toxic Substances and Disease Registry Case Studies in Environmental Medicine (CSEM) Cadmium Toxicity. US Department of Health Human Services Agency for Toxic Substances, Disease Registry Division of Toxicology, Environmental Medicine, Educational Services Branch, Atlanta, 2008:10-18.
22. Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S. 2005. Chromium toxicity in plants. Environ Int. 31(5):739-753.
23. DesMarias TL, Costa M. 2019. Mechanisms of chromium-induced toxicity. Curr Opin Toxicol. 14:1-7.
24. Sajid Abdulabbas H, Mohamed H, Jawad Al-Imari M, Haider Al-Mawlah Y, Hashim Shaheed S. 2023. The genotypes of glutathione peroxidase 1 (GPx1) (Rs1050450) affect some biomarker levels in the breast cancer patients. J Biotech Res. 14:153-159.
25. Thomas DM, Ivanescu AE, Martin CK, Heymsfield SB, Marshall K, Bodrato VE, *et al*. 2015. Predicting successful long-term weight loss from short-term weight-loss outcomes: new insights from a dynamic energy balance model (the POUNDS Lost study). Am J Clin Nutr. 101(3):449-454.
26. Vafaeimanesh J, Parham M, Norouzi S, Hamednasimi P, Bagherzadeh M. 2018. Insulin resistance and coronary artery disease in non-diabetic patients: Is there any correlation? Caspian J Intern Med. 9(2):121-126.
27. Mohamed H, Haider Al-Mawlah Y, Sajid Abdulabbas H. 2023. Examination and analyzing the levels of related micronutrients and anemia in pregnant women. J Biotech Res. 14:35-40.
28. Hashim A, Harbi S, Burhan M, MAWLAH Y, Hadi A. 2023. Histological and physiological determinants of hypothyroidism in patients and its relationship with lipid profile. J Adv Biotechnol Exp Ther. 6:9-16.