Contents list available http://www.kinnaird.edu.pk/

Journal of Natural and Applied Sciences Pakistan

Journal homepage: http://jnasp.kinnaird.edu.pk/

EVALUATION OF THE BIOCHEMICAL PARAMETERS IN SERUM OF PATIENTS WITH CARDIOVASCULAR DISEASE: A CROSS-SECTIONAL STUDY FROM DIYALA, IRAQ

Hayder T Qaddoori[1*], Haider Kh. Abbas[1]

[1]Middle Technical University – Baqubah Technical College, Dayala- Iraq

Article Info

*Corresponding Author

Email: haydertawfeeq510@gmail.com

Abstract

This descriptive study was conducted in Diyala, Iraq, to compare health indicators between cardiac patients and healthy individuals. Samples were collected from Diyala Medical laboratories in Baquba. The study analyzed gender ratios and different age groups, with male patients comprising 62% compared to 60% in the control group, while female patients represented 38% compared to 40% in the control group. Participants' ages ranged from 20 to 60 years, with the highest concentration in the 40-49 and 50-59 age groups. Results showed a significant increase in levels of cholesterol, triglycerides, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and calcium in patients compared to the control group ($P<0.05$). A significant decrease in high-density lipoprotein (HDL) and vitamin B12 levels was also observed in patients.ROC curve analysis showed highest sensitivity for cholesterol, followed by triglycerides, VLDL, and LDL, while HDL and B12 showed the lowest sensitivity. Regarding specificity, HDL recorded the highest level, followed by LDL, cholesterol, and triglycerides, while VLDL showed the lowest specificity. The study revealed a relationship between lipid levels, vitamin B12, and calcium, indicating that cardiac patients are more susceptible to disturbances in these indicators.

Keywords

Lipid profile, B12, Calcium, Heart disease, LDL.

1. Introduction

Cardiovascular disease (CVD) represents one of the leading causes of mortality globally, accounting for approximately 17 million deaths in 2016 (David.P.I, *et al*. 2020). Multiple risk factors contribute to CVD development, including advanced age, obesity, central adiposity, tobacco use, sedentary lifestyle, hypertension, dyslipidemia, and coagulation abnormalities. These risk factors frequently correlate with insulin resistance syndrome, characterized by hyperinsulinemia (Evans, *et al*. 2020). Homocysteine (Hcy), a sulfur-containing amino acid, emerges through methionine metabolism following dietary protein consumption. Research has established hyperhomocysteinemia as an independent risk factor for atherothrombotic disease, though the underlying mechanistic pathways remain incompletely understood (Sagar, Rashmi *et al*. 2019, Celik, S.F., Celik, E.2018). The metabolism of homocysteine involves several B-vitamins as essential cofactors, particularly folate, vitamin B6, and vitamin B12 (Ahmed Jayedi, Mahdieh. 2019). Deficiencies in these vitamins, especially B12, can lead to elevated plasma homocysteine levels. Clinical studies have demonstrated a significant association between increased homocysteine concentrations and elevated cardiovascular risk (Ishihara Junko, *et al*. 2008, Gunn-Helen Moen, *et al*. 2018, and Steardo Jr, *et al*. 2020). Calcium serves as a crucial regulatory element in various physiological processes within the human body. This essential mineral demonstrates significant involvement in maintaining musculoskeletal homeostasis, orchestrating immune system responses, facilitating antioxidant functionality, and participating in diverse inflammatory pathways and mechanisms. The maintenance of calcium homeostasis is governed by an intricate endocrine system that orchestrates calcium flux across three primary physiological interfaces: the gastrointestinal epithelium, renal tubules, and bone matrix. This sophisticated regulatory mechanism is predominantly mediated by parathyroid hormone (PTH), which serves as the principal endocrine regulator of calcium homeostatic processes (Mei Chung, *et al*. 2016, Wu, F., Juonala, *et al*. 2019) Epidemiological research and interventional clinical studies have demonstrated a significant correlation between calcium supplementation and elevated cardiovascular morbidity and mortality rates in human populations. Longitudinal studies indicate that chronic high-dose oral calcium supplementation is associated with an increased propensity for adverse cardiovascular events. This association is particularly pronounced in cases of extended supplemental calcium intake as opposed to dietary calcium consumption (Ingles, David Perez, *et al*. 2020).

2. Mothed and Material

An investigation was conducted at the Diyala Medical Laboratory in Baquba, Iraq, in 2020. The investigation was carried out over the course of three months, specifically, in the months of September, October, and November. Overall, 80 samples were collected Diyala Medical laboratories for the study, with 50 samples from patients and 30 samples from healthy individuals. The samples were collected from a variety of different people.Blood samples were obtained by first extracting venous blood with a plastic medical syringe that had a capacity of 5 millilitres and then transferring the blood into test tubes that included a gel material (gel tube). A period of thirty minutes was provided for the tube to remain at room temperature to enhance coagulation of the blood. The blood was separated in a short amount of time via centrifugation.

The mixture was centrifuged for seven minutes at a speed of three thousand revolutions per minute. The centrifugation process lasted for five minutes. The serum was then extracted from the other components via a micropipette after this step was completed. After that, the serum was separated into equivalent amounts of 250 microliters each, and then it was placed into Eppendorf tubes of comparable size. To prevent the serum from being repeatedly thawed and frozen, the tubes were kept at a temperature of -20 degrees Celsius until they were used once or twice. These precautions were taken throughout the storage process. The Cobas 411 method was used to evaluate the biochemical parameters, which included the lipid profile, serum calcium level content, and vitamin B12 level content.

3. Results

Results showed significant gender distribution differences ($p<0.05$) between patient and control groups. Males comprised 62.0% of patient's vs 60.0% of controls, while females made up 38.0% of patients vs 40.0% of controls. The highest frequency age brackets were 40-49 and 50-59 years for both groups, while the lowest were 20-29 and 30-39 years for patients, and 30-39 and ≥60 years for controls. Regarding BMI, 60.0% of patients had BMI >25 compared to 43.3% of controls, while only 2.0% of patients and 0.0% of controls had BMI <25, representing a significant difference between groups (Table 1).

Table 1: Chi-square test and Student's t test comparisons of the anthropometric characteristics.

			Groups			
			Patients (N=50)	Control (N=30)	Total	P value
Sex	Male	N	31	18	49	0.032*
		%	62.0%	60.0%	61.3%	
	Female	N	19	12	31	
		%	38.0%	40.0%	38.8%	
Age periods	20-29	N	0	3	3	0.001***
		%	0.0%	10.0%	3.8%	
	30-39	N	9	0	9	
		%	18.0%	0.0%	11.3%	
	40-49	N	15	12	27	
		%	30.0%	40.0%	33.8%	
	50-59	N	14	14	28	
		%	28.0%	46.7%	35.0%	
	≥ 60	N	12	1	13	
		%	24.0%	3.3%	16.3%	
BMI	<25	N	1	17	18	0.002**
		%	2.0%	56.7%	22.5%	
	25	N	19	13	32	
		%	38.0%	43.3%	40.0%	
	>25	N	30	0	30	
		%	60.0%	0.0%	37.5%	

This study investigated differences between patient and control groups across multiple health parameters. The patient group (mean age 50.58±11.78 years) was significantly older than the control group (43.33±9.59 years; $p<0.05$). Body Mass Index (BMI) was markedly higher in patients (27.06±2.80) compared to controls (21.23±3.10; $p<0.05$). Lipid profiles showed significant differences, with patients exhibiting

elevated levels of cholesterol (260.80±34.92 vs 146.00±24.37), LDL (98.06±22.00 vs 60.07±9.26), triglycerides (192.68±38.52 vs 85.93±14.08), and VLDL (41.74±9.77 vs 17.63±4.96). Conversely, HDL levels were significantly lower in patients (39.06±5.18) compared to controls (55.07±6.44). All differences were statistically significant ($p<0.05$).

Table 2: Test Results.

Groups		N	Mean	SD.	P value
Age	Pat.	50.0	50.57	11.77	0.0091**
	Con.	30.0	43.34	9.58	
BMI	Pat.	50.0	27.07	2.81	0.0052**
	Con.	30.0	21.24	3.11	
Cholesterol	Pat.	50.0	260.81	34.91	0.0021**
	Con.	30.0	146.01	24.38	
HDL	Pat.	50.0	39.07	5.19	0.0013***
	Con.	30.0	55.08	6.45	
LDL	Pat.	50.0	98.07	22.01	0.0051**
	Con.	30.0	60.08	9.27	
Triglycerides	Pat.	50.0	192.69	38.51	0.0032**
	Con.	30.0	85.94	14.09	
VLDL	Pat.	50.0	41.75	9.76	0.0041**
	Con.	30.0	17.64	4.68	

Pat: Patient
Con: Control

The study revealed significant differences in both calcium and vitamin B12 levels between patient and control groups ($P<0.05$). Calcium levels were elevated in the patient group (11.48±2.66) compared to controls (9.47±1.74). Conversely, vitamin B12 levels were markedly lower in patients (148.34±34.45) versus controls (262.87±14.60). Both differences were determined to be statistically significant through comparative analysis.

Table 3: Comparison of Calcium and Vitamin B12 Levels between Patient and Control Groups.

Groups		N	Mean	Std. Deviation	P value
Calcium	Pat.	50	11.48	2.66	0.001***
	Con.	30	9.47	1.74	
B12	Pat.	50	148.34	34.45	0.002**
	Con.	30	262.87	14.60	

This study examined the sensitivity and specificity of various lipid parameters and vitamin B12. Total cholesterol demonstrated maximum sensitivity (100%), followed by triglycerides (99%), VLDL (97%), and LDL (94%). In contrast, HDL cholesterol and vitamin B12 showed minimal sensitivity at 2%

and 0%, respectively. Regarding specificity, HDL cholesterol exhibited the highest value at 76%, while LDL and total cholesterol both showed 53% specificity. Triglycerides had a specificity of 51%, and VLDL demonstrated the lowest specificity at 19%, as evidenced by the data presented in Table 4 and Figure 1.

Table 4: Sensitivity and specificity of the comparison parameters of the current study ROC curve.

Parameters	Sensitivity (%)	Specificity (%)
Cholesterol	100.0%	53.08%
HDL	2.0%	76.11%
LDL	94.0%	53.13%
Triglycerides	99.0%	51.05%
VLDL	97.0%	19.07%
Calcium	74.0%	35.09%
B12	0.0%	42.09%

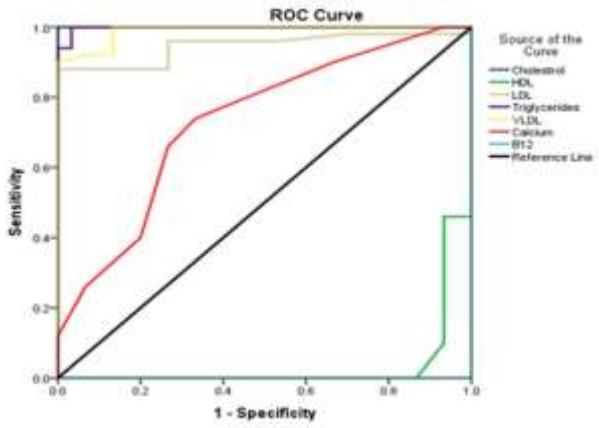

Figure 1: Specificity and sensitivity relationships according to the ROC curve.

The study revealed significant interrelationships among lipid profiles and key metabolic markers. Total cholesterol demonstrated positive correlations with low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), while exhibiting inverse relationships with other measured indices. High-density lipoprotein (HDL) showed negative associations with most indices, except for positive correlations with LDL, triglycerides, and calcium. LDL displayed positive correlations across all markers except vitamin B12, though notably demonstrating an inverse relationship with triglycerides. Triglycerides exhibited positive correlations with VLDL, calcium, and vitamin B12. Similarly, VLDL showed positive associations with both calcium and vitamin B12 levels. The data conclusively demonstrated a positive correlation between vitamin B12 and calcium levels.

Table 5: Pearson correlation was used to determine the associations between the current study's characteristics and patient characteristics.

		Cholesterol	HDL	LDL	Triglycerides	VLDL	Calcium
HDL	R	-.013					
	P	.930					
LDL	R	.085	.326*				
	P	.555	.021				
Triglycerides	R	-.017	.244	-.136			
	P	.908	.088	.345			
VLDL	R	.024	-.093	.091	.116		
	P	.869	.520	.531	.423		
Calcium	R	-.055	.109	.087	.065	.072	
	P	.706	.452	.546	.655	.617	
B12	R	-.088	-.142	-.060	.211	.161	.074
	P	.541	.325	.681	.142	.265	.609

4. Discussion

There is a strong correlation between age and the prevalence of coronary heart disease (CHD), since the incidence of this condition increases with increasing age in individuals. This can mostly be linked to the decreased flexibility of the arteries as well as the increased prevalence of the condition after the age of 40. [Boudi, F.B. 2014] As people age, the likelihood of experiencing difficulties with the endothelium of their blood vessels increases. The result of this is an increase in the thickness and rigidity of the arteries, which in turn contributes to the development of diseases and the generation of blood clots inside the arteries themselves. The ability of the endothelium of blood vessels to produce or release nitric oxide (NO), which allows blood vessels to widen and inflammation to be reduced, is diminished. This results in the formation of blood clots due to the absence of release, which causes the blood vessels to widen [North, B.J., Sinclair,. (2012)]. The findings of the current analysis are consistent with the findings of research carried out by [Bundy, Joshua D., *et al*. (2020) and Von Sarnowski *et al*. (2013)], which indicate that the prevalence of heart disease increases with increasing age. Consistent with previous studies, this study revealed a strong correlation between body mass index and arteriosclerosis, as did [Lee, Chi-Ho, *et al*. (2018)]. Additionally, changes in the renin-angiotensin-aldosterone system (RAAS), which cause vasoconstriction, likely explain the robust associations among obesity, hypertension, and diabetes. Compared with healthy controls, individuals who suffer from multiple sclerosis and high blood pressure are more likely to experience

this effect. An increased likelihood of developing hypertension is associated with a body mass index that is greater than the normal threshold. Individuals whose body mass index (BMI) falls within the normal range experience a disruption in equilibrium as a result of increased insulin resistance and decreased high-density lipoprotein levels. High-density lipoprotein (HDL) levels, elevated blood cholesterol levels, and hypertension are all factors that contribute to metabolic issues and the progression of cardiovascular disorders in people who already have heart conditions [Gierach, M., etal. (2014) and Papathanasiou, G., *et al*. (2015)]. A greater quantity of cholesterol in the blood can have negative implications and increase the likelihood of developing cardiovascular disease. A prior investigation carried out in by [Sanne A. E. Peters. (2016)] and colleagues yielded results that are consistent with the findings of the current investigation. It is located at the coordinates [Jane A. Leopold, Joseph L., (2008)]. An increase in the level of cholesterol leads to an increase in the formation of reactive oxygen species (ROS), which in turn lowers or hampers the generation of nitric oxide (NO), a chemical that has the ability to dilate blood vessels. As a consequence, elevated cholesterol levels are considered to contribute to an increase in blood pressure because of the accumulation of cholesterol. The accumulation of cholesterol in blood vessels, in conjunction with the accumulation of fatty substances, can lead to calcification and the development of hardness. The end result of this process is the constriction of blood arteries, which not only hinders blood flow but also increases the danger of blood clots and arterial hardening. The findings of the current study are consistent with those of the Izzat study[Izzat, Farah Haqqi., (2015)], which showed that individuals who suffer from heart ailments have higher total cholesterol levels than control individuals do, even though they have reached a satisfactory level of equilibrium[Khan,S.S., Ning, H., *et al*. (2018)]. An increase in triglyceride levels is associated with an increased risk of cardiovascular disease (CVD). An increased breakdown of lipids that have accumulated in fat cells is caused by the absence of insulin, which causes the lipase enzyme in fat cells to become active. As a consequence, a considerable amount of glycerol and fatty acids are discharged into the bloodstream. Triglycerides, phospholipids, and glycerol are all produced by the liver, and they are then carried into the bloodstream after they are produced. The activity of lipoprotein lipase (LPL), an enzyme that removes triglycerides from the blood, is hindered when there is an inadequate amount of insulin present in the body. As a consequence, there is an increase in the quantity of lipids present in the bloodstream[Rahman, Faisal, *et al*. (2018), Chamberlain, James J., *et al*. (2018) and Shahzad, Sumayya, *et al*. (2018). The findings of the current study are consistent with those of the Izzat study, which showed that individuals who suffer from heart issues have considerably higher levels of triglycerides than do those in the control group[Izzat, Farah Haqqi., (2015)]. Furthermore, these findings lend credence to the findings of Ismail's research, which showed that patients who have suffered from myocardial infarction (AMI) have higher levels of triglycerides than the study group that served as the control[Khan, Anmar A., *et al*. (2018)]. An increase in hepatic lipase activity and a decrease in LPL activity, the enzymes responsible

for triglyceride breakdown into glycerol and fatty acids, are responsible for the low HDL level. An increase in the concentration of triglycerides in the blood is usually accompanied by a decrease in the amount of HDL cholesterol present in the blood. When present in sufficient quantities, high-density lipoproteins have the ability to prevent the development of sclerotic plaques and function as antioxidants[Jialal, Ishwarlal., Gurdeep Singh. (2019), Adiels M., *et al*. (2008), and Dobiasova, M., *et al*. (2009)]. Furthermore, the findings of this study lend credence to the findings of the Izzat trial, which indicated a significant decrease in high-density lipoprotein levels among patients who were diagnosed with CHD while simultaneously keeping a reference group for the sake of comparison[Izzat, Farah Haqqi., (2015)]. According to the findings of Dobiasova's research, an increase in VLDL-c caused by elevated triglyceride levels is consistent with these findings. Calcium is a strong signal of cardiac discomfort and is also responsible for the negative changes that damage the tiny arteries of the heart, as revealed by multiple studies [Dobiasova, M., *et al*. (2009)]. Calcium accumulates and settles in the coronary arteries, which is a substantial indicator of cardiac discomfort. The field of study known as cardiology is centred on the measurement of calcium levels in the coronary arteries for the purpose of determining the health of the heart or identifying problems that are associated with the heart[Larsson, S. C., *et al*. (2017), and Foley, R. N.,, *et al*. (2008)]. The findings of the study that was carried out by Maryam and colleagues indicated that increased consumption of calcium, particularly through the use of dietary supplements that included calcium, increased the risk of experiencing a myocardial infarction[Moradi, Maryam, *et al*. (2020)]. The findings of the current research support the findings of the study that was carried out by Whelton and colleagues. This study indicated that a rise in blood calcium levels outside of the normal range is a factor that contributes to the development of atherosclerosis. This results in the accumulation of calcium within the plaques, which ultimately leads to the occlusion of specific parts of the coronary arteries[Whelton, S.P., *et al*. (2019)]. According to the findings of the present study, homocysteine should be considered a significant risk factor for CVD. The levels of vitamin B12 in the bloodstream affect the concentration of homocysteine that is found in the bloodstream. It is essential to keep in mind that homocysteine is classified as an amino acid. Scientists have asserted that the danger that this poses to the cardiovascular system is comparable to the danger that cholesterol poses. There is a strong association between increased levels of homocysteine in the bloodstream and an increased risk of heart attack, according to the findings of a number of studies that were conducted on certain population groups. Studies conducted on animals have provided additional evidence that homocysteine can damage the inner lining of arteries, which can ultimately result in the development of atherosclerosis[Zhao, J.V., Schooling,C.M (2017), and Chrysant, S. G., Chrysant, G. S. (2018)]. Methionine, which is another type of amino acid, is broken down, which results in the production of homocysteine, which is an amino acid. Methionine is a naturally occurring amino acid that plays an essential role in the formation of all proteins found in the human diet. Owing to the natural production of this toxic amino

acid within the body, it causes damage to blood vessels, which in turn leads to arterial hardening and obstruction, which may ultimately result in a stroke or a heart attack in terms of numbers, the value is[LI, Zhen *et al*. (2018)]. A deficiency in vitamin B12 is related to increased vulnerability to coronary artery disease and heart disease, as demonstrated by the findings of the current study, which match the findings of the study that was carried out by Zhen and colleagues[Li, Z., *et al*. (2018)]. This finding is also in agreement with the findings of the research carried out by Fei and colleagues, which indicated that a deficiency in vitamins is one of the causes of elevated levels of homocysteine. In total, patients were included [MA, Fei, *et al*. (2017)].

5. Conclusion

This research demonstrates significant variations in biochemical parameters between CHD patients and controls, emphasizing the critical role of comprehensive biochemical monitoring in disease management. The findings particularly highlight alterations in calcium and Vitamin B12 levels, alongside traditional lipid markers. These results underscore the necessity for a holistic approach to CHD risk assessment, incorporating both conventional lipid parameters and vitamin/mineral status. Further research is warranted to elucidate the mechanistic relationships between these parameters, particularly focusing on Vitamin B12 and calcium homeostasis, and to develop more sensitive diagnostic markers. These insights contribute to our understanding of CHD's biochemical profile and may guide future therapeutic strategies.

References

Adiels, M., Olofsson, S. O., Taskinen, M. R., & Borén, J. (2008). Overproduction of very low–density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. *Arteriosclerosis, thrombosis, and vascular biology*, 28(7), 1225-1236.

Boudi, F.B. (2014) . Overview of atherosclerosis . eMedicine Retrieved from http://emedicine.medscape.com\article\1950759- overview

Bundy, J. D., Ning, H., Zhong, V. W., Paluch, A. E., Lloyd-Jones, D. M., Wilkins, J. T., & Allen, N. B. (2020). Cardiovascular health score and lifetime risk of cardiovascular disease: the cardiovascular lifetime risk pooling project. Circulation*: Cardiovascular Quality and Outcomes*, 13(7), e006450.

Chamberlain, J. J., Johnson, E. L., Leal, S., Rhinehart, A. S., Shubrook, J. H., & Peterson, L. (2018). Cardiovascular disease and risk management: review of the American Diabetes Association Standards of Medical Care in Diabetes 2018. *Annals of internal medicine*, 168(9), 640-650.

Chrysant, S. G., & Chrysant, G. S. (2018). The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. *Expert review of cardiovascular therapy*, 16(8), 559-565.

David.P.I, Jose B.Cruz.R, Hernando.G. (2020). Supplemental Vitamins and Minerals for Cardiovascular Disease prevention and Treatment. *Current Cardiology Reports* (2020) 22:22. Doi.org/10.1007/s11886-020-1270-1.

Dobiasova, M., Stribrna, J., Sparks, D.L., Pritchard, P.H., and Frohlich, J.J. (2009). Cholesterol esterification rate in very Low density

Lipoprotein and Low density Lipoprotein depleted plasma and relation to high density Lipoprotein subspecies, sex, hyperlipidemia , and Coronary artery Disease . Arteriosclerosis, *Thrombosis, and Vascular Biology*, 11(1): 64-70.

Evans, C. E. L. (2020). Dietary fibre and cardiovascular health: a review of current evidence and policy. *Proceedings of the Nutrition Society*, 79(1), 61-67.

Foley, R. N., Collins, A. J., Ishani, A., & Kalra, P. A. (2008). Calcium-phosphate levels and cardiovascular disease in community-dwelling adults: the Atherosclerosis Risk in Communities (ARIC) Study. *American heart journal*, 156(3), 556-563.

Gierach, M., Gierach, J., Ewertowska, M., Arndt, A., and Junik, R. (2014). Correlation between Body Mass Index and Waist Circumference in patients With Metabolic Syndrome . ISRN Endocrinology, Article ID 514589, 6 pages.

Gunn-Helen Moen, Elisabeth Qvigstad, Kåre I Birkeland, David M Evans, Christine Sommer. (2018). Are serum concentrations of vitamin B-12 causally related to cardiometabolic risk factors and disease? A Mendelian randomisation study. *Am J Clin Nutr*. 2018 Aug 1;108(2):398-404. doi: 10.1093/ajcn/nqy101.

Ingles, D. P., Cruz Rodriguez, J. B., & Garcia, H. (2020). Supplemental vitamins and minerals for cardiovascular disease prevention and treatment. *Current cardiology reports*, 22, 1-8.

Ishihara, J., Iso, H., Inoue, M., Iwasaki, M., Okada, K., Kita, Y., ... & JPHC Study Group. (2008). Intake of folate, vitamin B6 and vitamin B12 and the risk of CHD: the Japan Public Health Center-Based Prospective Study Cohort I. *Journal of the American College of Nutrition*, 27(1), 127-136.

Izzat, Farah Haqqi. (2015) Highly sensitive reactive protein interleukin-C6 and fibrinogen as evidence of coronary heart disease. Master Thesis, College of Education for Pure Sciences, University of Diyala, Department of Biology.

Jayedi, A., & Zargar, M. S. (2019). Intake of vitamin B6, folate, and vitamin B12 and risk of coronary heart disease: a systematic review and dose-response meta-analysis of prospective cohort studies. *Critical reviews in food science and nutrition*, 59(16), 2697-2707.

Jialal, I., & Singh, G. (2019). Management of diabetic dyslipidemia: An update. *World journal of diabetes*, 10(5), 280.

Khan, A. A., Mundra, P. A., Straznicky, N. E., Nestel, P. J., Wong, G., Tan, R., ... & Meikle, P. J. (2018). Weight loss and exercise alter the high-density lipoprotein lipidome and improve high-density lipoprotein functionality in metabolic syndrome. *Arteriosclerosis, thrombosis, and vascular biology*, 38(2), 438-447.

Khan, S. S., Ning, H., Wilkins, J. T., Allen, N., Carnethon, M., Berry, J. D., ... & Lloyd-Jones, D. M. (2018). Association of body mass index with lifetime risk of cardiovascular disease and compression of morbidity. *JAMA cardiology*, 3(4), 280-287.

Larsson, S. C., Burgess, S., & Michaëlsson, K. (2017). Association of genetic variants related

to serum calcium levels with coronary artery disease and myocardial infarction. *Jama*, 318(4), 371-380.

Lee, C. H., Chan, R. S., Wan, H. Y., Woo, Y. C., Cheung, C. Y., Fong, C. H., ... & Lam, K. S. (2018). Dietary intake of anti-oxidant vitamins A, C, and E is inversely associated with adverse cardiovascular outcomes in chinese—A 22-years population-based prospective study. *Nutrients*, 10(11), 1664.

Leopold, J. A., & Loscalzo, J. (2008). Oxidative mechanisms and atherothrombotic cardiovascular disease. *Drug Discovery Today: Therapeutic Strategies*, 5(1), 5-13.Mar; 5(1): 5–13. doi: 10.1016/j.ddstr.2008.02.001.

Li, Z., Gueant-Rodriguez, R. M., Quilliot, D., Sirveaux, M. A., Meyre, D., Gueant, J. L., & Brunaud, L. (2018). Folate and vitamin B12 status is associated with insulin resistance and metabolic syndrome in morbid obesity. *Clinical Nutrition*, 37(5), 1700-1706.

Ma, F., Wu, T., Zhao, J., Ji, L., Song, A., Zhang, M., & Huang, G. (2017). Plasma homocysteine and serum folate and vitamin B12 levels in mild cognitive impairment and Alzheimer's disease: a case-control study. *Nutrients*, 9(7), 725.

Mei Chung, Alice M Tang, Zhuxuan Fu, Ding Ding Wang, Sydne Jennifer Newberry. (2016). Calcium Intake and Cardiovascular Disease Risk: An Updated Systematic Review and Meta-analysis. *Ann Intern Med*. 2016 Dec 20;165(12):856-866. doi: 10.7326/M16-1165.

Moradi, M., Mahdavi, M. M. B., & Nogourani, M. K. (2020). The relation of calcium volume score and stenosis of carotid artery. *Journal of Stroke and Cerebrovascular Diseases*, 29(1), 104493.

North ,B.J., and Sinelair, D.A. (2012) . The Intersection Between Aging and Cardiovascular Disease . *Circulation Research*, 110(8):1097-1108.

Papathanasiou, G., Zerva, E., Zacharis, I., Papandreou, M., Papageorgiou, E., Tzima, C., Georgakopoulos, D., and Evangelou, A. (2015) . Association of High Blood Pressure with Body Mass Index , Smoking and physical Activity in Halthy Young Adults . *The open Cardiovascular Medecine Journal*, 9:5-17.

Rahman, F., Blumenthal, R. S., Jones, S. R., Martin, S. S., Gluckman, T. J., & Whelton, S. P. (2018). Fasting or non-fasting lipids for atherosclerotic cardiovascular disease risk assessment and treatment?. Current atherosclerosis reports, 20, 1-6.

S F Celik , E Celik .(2018). Subclinical atherosclerosis and impaired cardiac autonomic control in pediatric patients with Vitamin B12 deficiency. *Niger J Clin Pract* . 2018 Aug;21(8):1012-1016. doi: 10.4103/njcp.njcp_345_17.

Sagar, R., Aggarwal, N., & Jose, N. (2019). A Cross-Sectional Study of Serum Folate and Vitamin B12 Levels in Psychiatric Inpatients. *The Primary Care Companion for CNS Disorders*, 21(5), 23219.

Sanne A E Peters , Yankuba Singhateh , Diana Mackay , Rachel R Huxley , Mark Woodward. (2016). Total cholesterol as a risk factor for coronary heart disease and stroke in women compared with men: A systematic review and meta-analysis. 2016 May;248:123-31. doi:

10.1016/j.atherosclerosis.2016.03.016. *Epub* 2016 Mar 15.

Shahzad, S., Hasan, A., Faizy, A. F., Mateen, S., Fatima, N., & Moin, S. (2018). Elevated DNA damage, oxidative stress, and impaired response defense system inflicted in patients with myocardial infarction. *Clinical and applied thrombosis/hemostasis*, 24(5), 780-789.

Steardo Jr, L., Luciano, M., Sampogna, G., Carbone, E. A., Caivano, V., Di Cerbo, A., ... & Fiorillo, A. (2020). Clinical severity and calcium metabolism in patients with bipolar disorder. *Brain sciences*, 10(7), 417.

Von Sarnowski, B., Putaala, J., Grittner, U., Gaertner, B., Schminke, U., Curtze, S., ... & Tatlisumak, T. (2013). Lifestyle risk factors for ischemic stroke and transient ischemic attack in young adults in the Stroke in Young Fabry Patients study. *Stroke*, 44(1), 119-125.

Whelton, S. P., Al Rifai, M., Dardari, Z., Shaw, L. J., Al-Mallah, M. H., Matsushita, K., & Nasir, K. (2019). Coronary artery calcium and the competing long-term risk of cardiovascular vs. cancer mortality: the CAC Consortium. *European Heart Journal-Cardiovascular Imaging*, 20(4), 389-395.

Wu, F., Juonala, M., Pahkala, K., Buscot, M. J., Sabin, M. A., Pitkänen, N., ... & Magnussen, C. G. (2019). Youth and long-term dietary calcium intake with risk of impaired glucose metabolism and type 2 diabetes in adulthood. *The Journal of Clinical Endocrinology & Metabolism*, 104(6), 2067-2074.

Zhao, J. V., & Schooling, C. M. (2017). Homocysteine-reducing B vitamins And ischemic heart disease: a separate-sample Mendelian randomisation analysis. European *Journal of Clinical Nutrition*, 71(2),267-273.