University of Thi-Qar Journal of Science (UTJsci)
E-ISSN:2709-0256, ISSN Print: 1991-8690 Volume (10), No.1, June 2023

Study the relationship between Selenium , Vitamin B_6 , B_{12} and hyperhomocysteine in patients with cardiovascular disease in Thi-Qar Governorate

Jannat Sattar Jabbar
Chemistry Department/College of Science, University of Thi-Qar
Thi-Qar/ Iraq
jannats@sci.utq.edu.iq

Mohammed A. Auda
Chemistry Department/College of Science, University of Thi-Qar
Thi-Qar/ Iraq
moh.ajah@sci.utq.edu.iq

***Abstract*— Cardiovascular diseases(CVD) which include ischemic heart disease, stroke, heart failure, peripheral arterial disease, and several other cardiac and vascular illnesses, are the world's leading cause of mortality and significantly lower life expectancy. The research included measuring and evaluating Homocysteine vitamin B_6 , B_{12} and Selenium for cardiovascular patients. Methods: This study was conducted at Nasiriya Heart Center ,Nasiriya General Hospital and Biochemistry Laboratory in the College of science University of Thi-Qar in the period between (October ,2022 to March, 2023) . In formed oral consent was taken for patients. The study included 88 subjects , ages between (40 – 70) years. The subjects were divided into two groups : Control group included (38) supposed approximately healthy subject, and Patients group included (50) patients with Heart disease. Cases excluded from subjects: Kidney diseases, Liver failure, Thyroid diseases Immunological diseases, Urinary tract infections, Acute joint diseases Pregnant women, Diabetes. Results: There was a significant increase in homocysteine concentration in the blood serum of the patients group compared to the control group. Significant decrease in the level of vitamin B6 , B12 and Selenium in the serum of patients compared to the control group. Conclusions: The study indicated that homocysteine was elevated in cardiovascular patients, which is a risk factor for heart disease. In addition, the levels of vitamins B_6, B_{12} and Selenium decreased against the increase in homocysteine through the negative relationship between homocysteine and vitamins B_6, B_{12} and Selenium.**

***Keywords*— Cardiovascular diseases, Homocysteine, Vitamin B_6 , Vitamin B_{12} , Selenium.**

I. INTRODUCTION

Cardiovascular diseases(CVD) are the world's leading cause of mortality and significantly lower life expectancy , which include ischemic heart disease, stroke, heart failure, peripheral arterial disease, and several other cardiac and vascular illnesses [1].

The heart functions as a pump, and blood vessels are pathways for blood and cells that deliver oxygen and nutrients to sustain the molecular processes required for vascular development and the functionality of various tissues [2].There are two categories of CVD risk factors: modifiable and non-modifiable. Age, gender, ethnicity, and genetic characteristics are a few examples of risk variables that cannot be changed. Modifiable risk variables include body mass, hypertension, fatty acid and lipoprotein levels, and smoking behavior. Health-promoting practices can help prevent or reduce modifiable risk factors. Exercise, a healthy diet, taking medicine, and quitting smoking can all lower a person's chance of having CVD [3].Although women typically have a lower incidence of CVD than males, a number of clinical evidences have shown that women have a greater rate of death and worse prognosis after an acute cardiovascular (CV) event [4]. Cardiovascular complication rates are decreasing in the context of multifactorial risk reduction with statins and other lipid-lowering medications, antihypertensive treatments, and antihyperglycemic treatment approaches, but they continue to be higher for patients with diabetes mellitus than for those without [5]. Homocysteine(Hcy) is a poisonous, non-protein amino acid that contains sulfur that is produced through the interconversion of the amino acids methionine and cysteine. Remethylation and trans-sulfuration are the two mechanisms used to metabolize homocysteine, respectively [6]. Homocysteine levels are determined via blood testing. Homocysteine levels between 4.4 and 10.8 mol per liter of blood are regarded as normal. Hyperhomocysteinemia is a medical disorder marked by abnormally high blood homocysteine concentrations above 15 mol/L [7].Its name, homocysteine, derives from the fact that it shares chemical properties with cysteine. Endothelial cell destruction, a reduction in vascular flexibility, and modifications to the hemostasis process are thought to be caused by homocysteinemia. Homocysteine levels that are higher could intensify the negative consequences of risk factors like smoking, high blood pressure, and lipid metabolism as well as encourage the onset of inflammation [8].

Homocysteine has gained a lot of study interest in relation to cardiovascular disease, and it has been noted as a potential risk factor for stroke, coronary vascular disease, ischemic heart disease, and other vascular occlusive illnesses [9]. It is now widely accepted that there is a link between Hcy and neurological issues like depression, Parkinson's disease, and Alzheimer's disease. It has also

https://doi.org/10.32792/utq/utjsci/v10i1.957

been demonstrated that 10% to 30% of Parkinson's disease patients have elevated plasma total homocysteine (tHcy) levels[10].Hyperhomocysteinemia produces hypertension and cardiovascular disease is through homocysteine mediated damage to vascular smooth muscle and endothelial cells. This damage, in turn, leads to a loss of arterial vasodilation, vascular integrity, and thus increased blood pressure (BP) and accelerated atherosclerosis [11]. The human body needs vitamin B_6 to be healthy and function properly. It is made up of a collection of six chemical substances that are soluble in water [12]. The body employs vitamin B_6 in a variety of enzymatic processes, such as the synthesis of neurotransmitters, amino acid metabolism, glucose metabolism, lipid metabolism, hemoglobin synthesis function and gene expression [13].The vitamin is made up of a collection of six chemically linked substances, each of which has a pyridine ring at its core. They differ from one another by a changeable group at the 4′ position of the pyridine, which can be either an amino methyl group (pyridoxamine (PM)), a hydroxyl methyl group (pyridoxine (PN)), or an aldehyde (pyridoxal (PL)). The various derivatives can serve as Co-factors once they have been phosphorylated,with pyridoxal 5′-phosphate (PLP) being the physiologically active form of vitamin B_6 [14]. More than 150 enzymatic processes use the active form of vitamin B_6, pyridoxal 5′-phosphate (PLP),as a Co-factor [15]. Humans get their vitamin B_6 through their diet and their gut bacteria. B_6 is largely present in fruits and vegetables as pyridoxine, along with its phosphate and glucose. It mostly appears as PLP and PMP in meat and seafood [16].Since the human body cannot store B_6, a daily source is necessary [17].The daily recommended intake for individuals of vitamin B_6 is 1.3 mg. However, PLP levels appear to be decreased in a number of conditions, including intoxication, obesity, and pregnancy. A few pathogenic conditions, such as chronic renal insufficiency, end-stage renal disorders, and other kidney conditions, can also lower vitamin B_6 levels. In addition, vitamin B_6 insufficiency has been connected to malabsorption syndromes such celiac disease and inflammatory bowel diseases, as well as other malabsorption disorders [18]. Low plasma vitamin B_6 concentrations are not only linked to a higher incidence of atherosclerotic disease and, more particularly, CAD, but the higher risk also seems to exist independently of other known risk factors for CAD, such as homocysteine. Furthermore, appropriate vitamin B_6 levels were found to be a CAD prevention factor [19].

Cobalamin, often known as vitamin B_{12}, is a necessary water-soluble vitamin that is needed for regulating haematopoiesis and the health of neurons [20].The bacteria that live in the human body create vitamin B_{12} for us. Since it is primarily obtained from the consumption of meat products, vegetarians often have a deficiency by nature. This specific vitamin deficiency can cause weakness, weariness, constipation, balance problems, mental fogginess, peripheral tingling, depression and cognitive problems [21].

One-carbon metabolism requires vitamin B_{12} to function. It participates in the synthesis, methylation, and repair of DNA as well as the creation of cellular energy and epigenetic regulation processes. Since vitamin B_{12} is concentrated in animal tissues, diets containing animal products are the only sources of this vitamin. Liver, beef, lamb, chicken, eggs, dairy products and other foods are rich sources of vitamin B_{12}. A lack of animal foods in the diet or malabsorption of the vitamin are the main causes of vitamin B_{12} insufficiency. According to several studies, vitamin B_{12} deficiency is more prevalent in obese people, including obese children and teenagers, obese women with polycystic ovarian syndrome and obese pregnant women [22]. Lack of vitamin B_{12} has been linked to an increased risk of metabolic disorders in pregnancy, including fatty acids, GDM and insulin resistance [23]. The richest sources of cobalami6n are the heart, kidney and liver in particular. The commonly utilized cyano-form of vitamin B_{12} has a molecular weight of 1355 daltons, making it one of the largest and most structurally complicated nonpolymeric macromolecules yet described in nature. One central cobalt (Co) atom is coupled with four equatorial nitrogen atoms provided by pyrrole residues to form the corrinoids, a class of molecules known collectively as cobalamins [24]. Hyperhomocysteinemia is caused by a vitamin B_{12} deficiency. Elevated homocysteine (Hcy) is regarded as a stand-alone risk factor for CVD since it is connected to arterial endothelial dysfunction. Perhaps the most important way that low vitamin B_{12} status increases risk of CVD is via its role in Hcy metabolism [25].

Selenium is a crucial tracelement needed for a variety of biological processes. At least 25 human selenoproteins include selenium, and more specifically the amino acid selenocysteine, which is involved in a wide range of crucial biological processes, from the control of reactive oxygen species (ROS) concentration to the synthesis of hormones [26]. The most essential micronutrient for both humans and animals is selenium. Inorganic selenium is mostly deposited in plants through the sulfate absorption pathway; animals and humans later consume these sources as vegetables, meats, and nutritional supplements.For adult males and females, the recommended selenium consumption is 55 μg and 70 μg daily, respectively [27]. Supplementing with selenium can increase the body's resistance to oxidation, cancers, heavy metals, and harmful microbes as well as its immunity and fertility. Due to selenium's dual nature, excessive supplementation can have negative consequences on the body's ability to grow and develop, and in certain situations, it can even be fatal [28]. Biological forms of organic or inorganic selenium can be found in nature and in living things. Selenmethionine and selenocysteine are the two primary organic forms (Secys). The inorganic forms of selenium are selenite $(SeO_3)^{-2}$, selenide (S^{-2}), selenate $(SeO_4)^{-2}$ and selenium element (Se) [29]. It's also necessary to remember that before the creation of SeCys, which is essential in the development of selenoproteins, both inorganic and organic forms must first be transformed to inorganic H_2Se [30]. The element Se is crucial for maintaining cardiac health. Both low and excessive levels of Se can have harmful effects on cardiovascular health, thus it's critical to keep them at a healthy level in the body . Heart failure and cardiomyopathies like Keshan disease (KD) have both been associated to Se deficiency. Conversely, excessive consumption of selenium may cause deadly heart symptoms and severe poisoning [31]. By measuring glutathione peroxidase(GPx) activity in blood, it

is possible to demonstrate the elevated CVD risk connected to reduced Se consumption [32].

Aim of the study: The measurement of homocysteine, B_6, B_{12} and Selenium to Cardiovascular patients.

II. MATERIALS AND METHODS

A. Study design

This study was conducted at Nasiriya Heart Center , Nasiriya General Hospital and Biochemistry Laboratory in the College of science University of Thi-Qar in the period between (October ,2022 to March 2023) .The study included 88 men and women, 38control subjects and 50 patients their ages were between 40 – 70 years. Excluded cases from this: Kidney diseases, Liver failure, Thyroid diseases Immunological diseases, Urinary tract infections, Acute joint diseases Pregnant women, Diabetes.

B. Blood Sample collection

About (5ml) of blood was collected from men & women by venous . Placed in a gel tube to separate the serum. The blood was allowed to clot at room temperature , and then the serum was separated by centrifugation (10 min at 3000 xg) the serum samples was removed and stored at (-20°C) for later measurement biochemical parameters, unless used immediately.

C. Chemicals

The chemicals that were used in the study with the company are listed in the table below:

No.	Chemicals	Supplied Company & Country
1	Homocysteine Kit	Bioassay Technology Laboratory, china
2	Vitamin B_6 Kit	Bioassay Technology Laboratory, china
3	Vitamin B_{12} Kit	Bioassay Technology Laboratory, china
4	Nitric acid	BDH, England
5	Perchloric acid	BDH, England
6	Hydrochloric acid	BDH, England

These kits were measured by the ELISA device and concentration of element selenium in serum samples were measured by flame atomic absorption method.

D. The statistical analysis

All statistical analysis was performed using SPSS, Windows version 23.0 software and Microsoft Excel 2010. The results were expressed as mean± standard deviations (mean ± SD) ,and Least Significant Difference (LSD). One - way analysis of variance (ANOVA) was used to compare parameters in different studied groups.

III. RESULTS AND DISCUSSION

A. Homocysteine:

Table (1) shows a significant increase homocysteine in blood serum concentration in the patients group compared with the control group. Studies have revealed a strong link between hyperhomocysteinemia and cardiovascular disease, as well as its consequences, such as heart attacks and strokes [33].

Reduced nitric oxide production and coronary microvascular dysfunction make excessive serum homocysteine levels associated with an increased risk of major adverse cardiovascular events [34]. Potential mechanisms of Hcy's negative effects include endothelial dysfunction and mortality, increased oxidative stress, inflammation, and altered collagen metabolism [35]. Studies show a strong link between Hcy and CHD, and as a risk factor, it can be utilized to diagnose CHD on its own. The body experiences pre-thrombosis due to homocysteine's harmful effects on vascular endothelial cells, which include endothelial damage and malfunction, vascular smooth muscle cell proliferation, disruption of the balance between coagulation and fibrinolysis, and induction of pre-thrombosis [36].

From the early evidence, Hcy not only impacts endothelial function to result in a prothrombotic environment, but also initiates an including the N-methyl-D-aspartate receptor (NMDAr), reactive oxygen species (ROS), extracellular signal-regulated kinase (ERK), and nuclear factor kappa B (NF-KB) signal pathway, to cause an inflammatory response and speed up atherosclerosis in cardiovascular diseases [37].

Table (1): Serum homocysteine levels of control and patients groups

Groups	**NO.**	**HCY (pmol/mL) Mean ± SD.**
Patients	50	415.95±83.48
Control	38	83.17±22.17
P. value		**< 0.001**

B. Serum Vitamin B_6 Concentration

Table (2) show a significant decrease in the concentration of serum vitamin B_6 in patients group in comparison with controls group ($p< 0.001$). The results were consistent with previous studies [38] [12]. Homocysteine metabolisms have been shown to be influenced by vitamin B_6 . The vitamin B_6 may be linked to CVDs, according to studies [39]. Prior research has looked at the potential molecular processes that underlie the protective benefits of vitamin B_6 on the avoidance of CVD, including the suppression of lipoperoxide synthesis and reductions in homocysteine and inflammation, all of which are recognized CVD risk factors.Moreover, hypohomocysteinemia brought on by vitamin B_6 deficiency may result in arterial wall damage [40].Cardiovascular illnesses (CVD), stroke, diabetes, and cancer have all been proven to be often linked to low vitamin B_6 levels . By reducing inflammation and oxidative stress, supplemental vitamin B_6 consumption is thought to have anti-inflammatory and anti-inflammatory actions against such chronic illnesses [41].

Table (2): Serum Vitamin B_6 levels of control and patients groups

Groups	NO.	B_6 (nmol/l) Mean ± SD.
Patients	50	22.07±7.08
Control	38	93.26±27.28
P. value		**< 0.001**

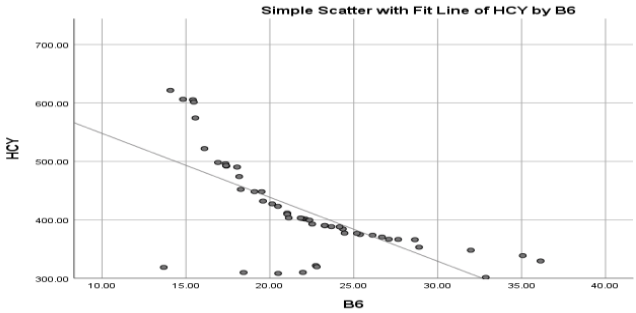

Figure (1): shows negative correlation between serum homocysteine and Vitamin B_6 with correlation coefficient (r= - 0.293 ,p-value = 0.039)

C. Serum Vitamin B_{12} Concentration

Table (3) show a significant decrease in the concentration of serum vitamin B_{12} in patients group in comparison with controls group (p< 0.001). The results were consistent with previous studies [42] [43]. Hyperhomocysteinemia is caused by vitamin B_{12} deficiency. Homocysteine (Hcy) levels that are elevated are linked to arterial endothelial dysfunction and are regarded as separate risk factors for CVD. In addition to deadly and non-fatal coronary disease, myocardial infarction, stroke, and other circulatory health issues, macrocytosis linked to vitamin B_{12} deficiency is also linked to these conditions. A lack of vitamin B_{12} may raise the risk of circulatory health issues in addition to the hyperhomocysteinemia that is brought on by this nutrient's role in macrocytosis [25]. B_{12} may possess antioxidant properties , and subclinical B_{12} deficiency may thus contribute to oxidative stress and the onset of age-related diseases. Since hepatic B_{12} stores vastly exceed the daily loss of the vitamin, deficiencies can remain clinically unexpressed for years . In the absence of genetic causes, subclinical B_{12} deficiency can be caused by three general factors: inadequate intake, increased demand, and malabsorption [20]. Hcy accumulates if conversion to methionine is slowed because of a shortage ofvitamin B_{12}, and a raised plasma Hcy suggests sub-optimal nucleic acid and amino-acid metabolism. It also has direct harmful effects, e.g., it increases the risk of cardiovascular disease through thickening the lining of blood vessels and may also increase the risk of certain cancers and dementia. Vitamin B_{12} deficiency can also lead to hyperhomocysteine (HHcy) and may be associated with osteoporosis, depression, cognitive decline, and some forms of dementia in the elderly [44].

Table (3): Vitamin B_{12} levels in serum of control and patients groups

Groups	NO.	B_{12} (pg/ml) Mean ± SD.
Patients	50	84.65±15.82
Control	38	136.36±33.51
P. value		**< 0.001**

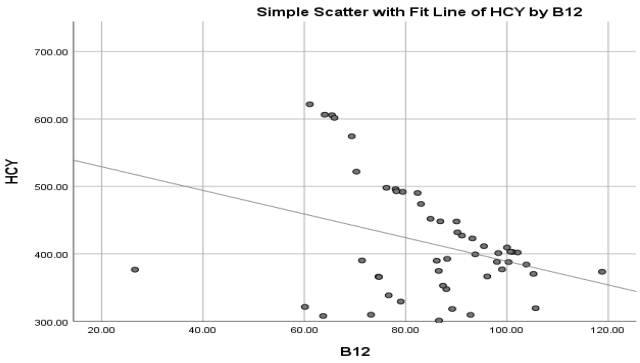

Figure (2): shows negative correlation between serum homocysteine and Vitamin B_{12} with correlation coefficient(r= - 0.099 ,p-value = 0.492)

D. Serum Selenium Concentration

Table (4) show a significant decrease in the concentration of serum Selenium in patients group in comparison with controls group (p< 0.001). Cardiovascular illnesses such as myocardial infarction, heart failure, and cardiomyopathies like Keshan's disease (KD) have all been related to Se deficiency. Although there is mounting evidence that selenium is crucial for healthy cardiovascular function, little is known about how selenium interacts with cardiovascular disorders, particularly when there is dietary selenium insufficiency, On the other hand, excessive Se use could lead to deadly cardiac symptoms and severe poisoning [31]. Selenium plays an essential part in the selenoprotein-induced defense system. Consequently, selenium blood levels have been widely utilized as a biomarker for oxidative stress-associated diseases. Various observational studies have investigated the significance of serum selenium levels on the development of cardiovascular diseases . Oxidative stress plays a pivotal role in the chronic as well as the acute phase of coronary heart disease (CHD) [45]. Oxidative stress is involved in the etiology of several chronic diseases, including CVD, diabetes, and stroke . Stress can disrupt homeostasis, leading to inflammation and apoptosis in cells. The increased CVD risk associated with low Se intake can be demonstrated by evaluating glutathione peroxidase (GPx) activity in blood. The absence of GPx can accelerate the occurrence of atherosclerosis, which is a risk factor CVD [32].

Table (4): Serum Selenium levels of control and patients groups

Groups	NO.	Se (µg/ml) Mean ± SD.
Patients	50	47.68±11.72
Control	38	139.92±29.85
P. value		**< 0.001**

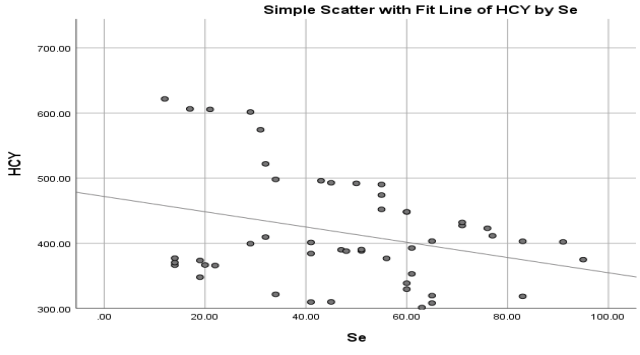

Figure (3):shows negative correlation between serum homocysteine and Selenium with correlation coefficient (r= - 0.021 ,p-value = 0.827)

IV. CONCLUSIONS

The study indicated that homocysteine was elevated in cardiovascular patients, which is a risk factor for heart disease. In addition, the levels of vitamins B_6, B_{12} and Selenium decreased against the increase in homocysteine through the negative relationship between homocysteine and vitamins B_6, B_{12} and Selenium**.**

REFERENCES

[1] G. A. Mensah, G. A. Roth, C. O. Johnsonm, G. Addolorato, E. Ammirati, L. M. Baddour, and C. B. Noël, "Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study, " vol. 76, no. 25, 2020.

[2] F. Paganelli, G. Mottola, J. Fromonot, M. Marlinge, P. Deharo, R. Guieu, and J. Ruf, "Hyperhomocysteinemia and cardiovascular disease: is the adenosinergic system the missing link?," vol. 22, no. 4, P. 8, 2021.

[3] C. C. Mes, and F. M. Lewis, "Family history of cardiovascular disease, perceived cardiovascular disease risk, and health-related behavior: a review of the literature," vol. 29, no. 2, P. 1, 2014.

[4] Z. Gao, Z. Chen, A. Sun , and X. Deng, "Gender differences in cardiovascular disease. Medicine in Novel Technology and Devices," vol. 4, P. 12, 2019.

[5] C. C. Low Wang, C. N. Hess, W. R. Hiatt, and A. B. Goldfine, "mellitus: atherosclerotic cardiovascular disease and heart failure in type 2 diabetes mellitus–mechanisms, management, and clinical considerations," vol.133, no. 24, P.14, 2016.

[6] T. Hasan, R. Arora, A.K. Bansal, R. Bhattacharya,G. S. Sharma, L. R. Singh, "Disturbed homocysteine metabolism is associated with cancer. Experimental & molecular medicine," vol. 51, no. 2, P. 21, 2019.

[7] T. Rujaswini, D. Praveen, P. R. Chowdary, M. V. Aanandhi, and P. Shanmugasundaram, "A review on association of serum homocysteine in diabetic neuropathy," vol. 10, no. 2, 2018.

[8] M. A. Khaleefah, I. I. Latif, A. A. Jaffer, "Homocysteinemia in Heart Failure," vol. 20, no. 1, P.15, 2021.

[9] J. Xu, X. Zhao, S. Sun, P. Ni, C. Li, A. Ren, and L. Zhu, "Homocysteine and digestive tract cancer risk: a dose-response meta-analysis," vol. 2018, P. 18, 2018.

[10] L. Hannibal, and H. J. Blom, "Homocysteine and disease:Causal associations or epiphenomenons? " vol. 53, 2017.

[11] M. Hammed, and M. Ajah, "The relationship of Homocystein and some Biochemical parameters in hypertensive Patients from Thi-Qar Governorate," University of Thi-Qar, College of Science, 2022.

[12] K. Stach, W. Stach, and K. Augoff, "Vitamin B_6 in health and disease," vol. 13, no. 9, P. 17, 2021.

[13] A. Hemminger, and B. K. Wills, "Vitamin B_6 Toxicity," PMID: 32119387, P. 3, 2020.

[14]M. Parra, S. Stahl, and H. Hellmam, "Vitamin B_6 and its role in cell metabolism and physiology," vol. 7, no. 7, P. 22, 2018.

[15] P. M. Ueland, A. McCann, Ø. Midttun, and A. Ulvik, "Inflammation, vitamin B_6 and related pathways," vol. 53, 2017.

[16] M. P. Wilson, B. Plecko, P. B. Mills, and T. P. Clayton, "Disorders affecting vitamin B_6 metabolism," vol. 42, no. 4, P. 22, 2019.

[17] M. J. Brown, M. A. Ameer, and K. Beier, "Vitamin B_6 deficiency," PMID: 29261855, P. 22, 2017.

[18] E. Mascolo, and F. Verni, "Vitamin B_6 and diabetes: relationship and molecular mechanisms," vol. 21, no. 10, P. 23, 2020.

[19] V. Lotto, S. W. Choi, and S. Friso, "Vitamin B_6: a challenging link between nutrition and inflammation in CVD," vol.106, no.2, P. 13, 2011.

[20] E. E. van de Lagemaat, L. C. de Groot, and E. G. van den Heuvel, "Vitamin B_{12} in relation to oxidative stress: a systematic review," vol. 11, no. 2, P. 25, 2019.

[21] P. Sangle, O. Sandhu, Z. Aftab, A. T. Anthony, and S. Khan, " Vitamin B12 supplementation: preventing onset and improving prognosis of depression," vol. 12, no. 10, P. 26, 2020.

[22] Y. Sun, M. Sun, B. Liu, Y. Du, S. Rong, G. Xu, and W. Bao, "Inverse association between serum vitamin B_{12} concentration and obesity among adults in the United States," vol. 10, 2019.

[23] X. Chen, Y. Zhang, H. Chen, Y. Jiang, Y. Wang, D. Wang, and W. Yan, " Association of Maternal Folate and Vitamin B_{12} in Early Pregnancy With Gestational Diabetes Mellitus: A Prospective Cohort Study," vol. 44, no. 1, P. 6, 2021.

[24] A. Sobczyńska-Malefora, E. Delvin, A. McCaddon, K. R. Ahmadi, and D. J. Harrington, "Vitamin B12 status in health and disease: a critical review. Diagnosis of deficiency and insufficiency – clinical and laboratory pitfalls," vol. 58, no. 6, P. 21, 2021.

[25] R. Pawlak,"Is vitamin B12 deficiency a risk factor for cardiovascular disease in vegetarians," vol. 48, no. 6, P. 18, 2015.

[26] G. Barchielli, A. Capperucci, and D. Tanini, "The role of selenium in pathologies: An updated review. Antioxidants,"vol. 11, no. 2, P. 27, 2022.

[27]S. Hannibal, and S. Dharmaraj, "Selenium and selenoproteins: It's role in regulation of inflammation. Inflammopharmacology," vol. 28, P. 6, 2020.

[28] Q. Lv, X. Liang, K. Nong, Z. Gong, T. Qin, X. Qin, and Y. Zhu, "Advances in research on the toxicological effects of selenium," vol. 106, P. 6, 2021.

[29] Y. Mehdi, J. L. Hornick, L. Istasse, and I. Dufrasne, "Selenium in the environment, metabolism and involvement in body functions," vol.18, no. 3, P. 13, 2013.

[30] L. H. Duntas,"Selenium and at-risk pregnancy: challenges and controversies. Thyroid research," vol. 13, no. 1, P. 1, 2020.

[31] B. K. Shimada, N. Alfulaij, and L. A. Seale, "The impact of selenium deficiency on cardiovascular function, " vol. 22, no. 19, P. 2, 2021.

[32] A. Shalihat, A. N. Hasanah, R. Lesmana, A. Budiman, and D. Gozali, "The role of selenium in cell survival and its correlation with protective effects against cardiovascular disease: A literature review," vol. 134, 2021.

[33] P. Ganguly, and S. F. Alam, "Role of homocysteine in the development of cardiovascular disease," vol. 14,no. 1, P. 10, 2015.

[34] A. Ahmed, M. T. Corban, T. Toya, J. D. Sara, B. Lerman, J.Y. Park and A. Lerman, "Coronary microvascular endothelial dysfunction in patients with angina and nonobstructive coronary artery disease is associated with elevated serum homocysteine levels. Journal of the American Heart Association, " vol. 9, no. 19, P. 30, 2020.

[35]N. Jin, L. Huang, J. Hong, X. Zhao, Y. Chen, J. Hu, and J. Pu, "Elevated homocysteine levels in patients with heart failure: A systematic review and meta-analysis," vol. 100, no. 33, P. 20, 2021.

[36] Z. Zhang, L. Wang, Y. Zhan, C. Xie, Y. Xiang, D. Chen, and Y. Wu, "Clinical value and expression of Homer1, homocysteine, S-adenosyl-l-homocysteine, fibroblast growth factors 23 in coronary heart disease," vol. 22, no. 1, P.12, 2022.

[37] Y. Zhao, and J. Zhang, "Clinical implication of homocysteine in premature acute coronary syndrome female patients: Its distribution and association with clinical characteristics and major adverse cardiovascular events risk," vol. 100, no. 18, P. 7, 2021.

[38] S. Friso, V. Lotto, R. Corrocher, and S. W. Choi, "Vitamin B_6 and cardiovascular disease. Water Soluble Vitamins: Clinical Research and Future Application," vol. 56, no. 2, 2012.

[39] J. Wei, and S. J. Ji, "Modification of vitamin B_6 on the associations of blood lead levels and cardiovascular diseases in the US adults," vol. 3, no. 2, P. 12, 2020.

[40] J. Jeon, and K. Park, "Dietary vitamin B6 intake associated with a decreased risk of cardiovascular disease: A prospective cohort study," vol. 11, no. 7, P. 29, 2019.

[41] T. Kumrungsee, P.Zhang, N. Yanaka, T. Suda, and N. Kato, "Emerging cardioprotective mechanisms of vitamin B_6: a narrative review," vol. 61, no. 2, P. 26, 2022.

[42] H. N. Kim, Y. M. Eun, and S. W. Song, "Serum folate and vitamin B_{12} levels are not associated with the incidence risk of atherosclerotic events over 12 years: the Korean Genome and Epidemiology Study," vol. 63, Pages 34-41, 2019.

[43] I. Capelli, G. Cianciolo, L. Gasperoni, F. Zappulo, F. Tondolo, M. Cappuccilli, and G. La Manna, "Folic acid and vitamin B_{12} administration in CKD, why not?. Nutrients, "vol. 11, no. 2, P. 13, 2019.

[44] E. Azzini, A. Raguzzini, and A. Polito, " A brief review on vitamin B_{12} deficiency looking at some case study reports in adults. International journal of molecular sciences," vol. 22, no. 18, P. 7, 2021.

[45]C. Benstoem, A. Goetzenich, S. Kraemer, S. Borosch, W. Manzanares, G. Hardy, and C. Stoppe,"Selenium and Its Supplementation in Cardiovascular Disease What do We Know? ," vol.7, no. 5, P. 27, 2015.