HOMOCYSTEINE AS A CARDIOVASCULAR RISK FACTOR

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Abstract: This article aims to review the evidence on the relationship between homocysteine and cardiovascular disease (CVD), as well as the possible mechanisms involved and therapeutic strategies to reduce homocysteine levels. Homocysteine is an amino acid that can accumulate in the blood for various reasons, such as enzymatic defects, nutritional deficiencies, or changes in liver or kidney function. Hyperhomocysteinemia is considered an independent risk factor for CVD as it affects the vascular endothelium, promotes LDL oxidation, and stimulates thrombosis. The article presents a meta-analysis of clinical and experimental studies that investigated the association between homocysteine and CVD, the mechanisms by which homocysteine can cause vascular damage, and ways to treat hyperhomocysteinemia, mainly through supplementation with B vitamins. The article concludes that homocysteine is both a marker and a causal factor of CVD, and that reducing its levels can prevent or slow the progression of the disease.

Keywords: Cardiovascular Diseases; Homocysteine; Diseases; Markers.

INTRODUCTION

Among the numerous markers that have been investigated in the literature to assess the probability of developing cardiovascular disease (CVD) (Habib, et al; 2023), homocysteine stands out as one of the independent, non-traditional and debatable risk factors for arterial disease. coronary disease (Hankey, Eikelboom, 1999). Homocysteine is an amino acid formed from the conversion

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of methionine to cysteine (Faeh, et al., 2006). Significant elevations in homocysteine levels can be observed in patients with defects or mutations in the metabolic enzymes involved in this process, such as cystathionine β-synthase and 5,10-methylenetetrahydrofolate reductase (MTHFR) (Faeh, et al., 2006). Homocysteine elevations are found in individuals with vitamin B12 or folic acid deficiencies, although these changes can be modified (Veeranna, et al., 2011). Other factors that may result in hyperhomocysteinemia include methotrexate overdose/toxicity or impaired liver or kidney function (Hankey, Eikelboom, 1999).

The main concern with high levels of circulating homocysteine is its impact on the endothelial cells lining blood vessels. Homocysteine also interferes with the oxidation of low-density lipoproteins and has prothrombotic properties (Baszczuk, Kopczyński, 2014). It is known that many patients with CVD have high levels of non-traditional risk factors, including homocysteine (Shenoy, et al., 2014).

Atherosclerotic cardiovascular diseases (ASCVD) increasingly affect young individuals around the world (Ichikawa, et al., 2023). This is a situation with a major impact on public health, as people with ASCVD may lose the ability to work and have greater health expenses throughout their lives.

Some traditional and well-established risk factors for CVD are smoking, diabetes, hyperlipidemia, hypertension, metabolic syndrome, prothrombotic states, and chronic inflammation (Hankey, Eikelboom, 1999). Smoking is a modifiable risk factor that increases the likelihood of peripheral arterial disease and abdominal aortic aneurysm by five times, and two to three times the likelihood of CVD, stroke and sudden cardiac death (Shenoy, et al., 2014). However, the presence of multiple traditional and non-traditional risk factors may generate inconsistent results on the association between homocysteine and CVD.

The purpose of this systematic review is to synthesize the available evidence on the role of homocysteine in ASCVD in young adults and children. These findings will allow us to identify which pathological conditions, patient characteristics, medications/interventions and biomarkers influence homocysteine levels and, consequently, the risk of CVD. These findings may be applied in clinical

practice to guide appropriate management based on homocysteine levels obtained in laboratory tests.

METHODOLOGY

Search strategy

This review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Figure 1). The article selection period was from 2013 to 2023. The databases searched were PubMed and SciELO.

The search strategy involved the use of specific keywords in combination with the conjunctions "OR" and "AND". These keywords included "coronary artery disease," "coronary heart disease," "coronary heart disease," "vascular disease," "atherosclerosis," "arteriosclerosis," and "homocysteine." In the second phase, two independent reviewers examined the search results; Initially, they reviewed the titles and abstracts and excluded any studies that were not relevant.

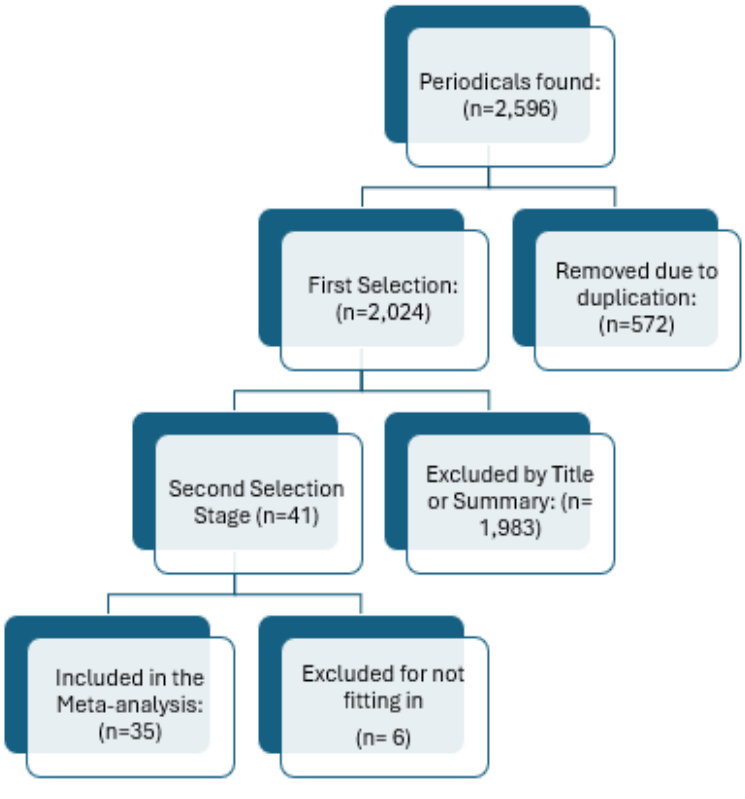
Abstracts that were related to our topic were considered for a full review. The full texts of all pertinent articles and those requiring further study were then obtained and rechecked against eligibility criteria. A total of 2,596 studies were screened. After pre-screening and removing all duplicates, 572 studies were eliminated from the total of 2,596. Titles and abstracts were then reviewed by two independent investigators to include only those studies relevant to the topic of interest. At this stage, around 1,983 studies were excluded. All remaining 41 studies were carefully reviewed for inclusion criteria and there were 6 studies that did not meet them. Finally, 35 studies were included in the final analysis of the systematic review. Details are mentioned in Figure 1 which shows the flow diagram of the entire article selection process as per PRISMA guidelines.

Inclusion and exclusion criteria

Inclusion criteria were studies that included homocysteine as a cause of premature myocardial infarction or coronary artery disease for patients under 45 years of age, regardless of gender. Case-control, cohort and cross-sectional studies of human subjects published in English were included.

Exclusion criteria were studies published before January 2013 and after 2023. Conference abstracts, review articles, research theses, editorials, commentaries, opinions, views, case reports and systematic reviews were all excluded. Duplicates and retracted articles that did not meet the inclusion criteria were automatically filtered.

Figure 1: Identification of studies via databases and records



Source: The author (2024)

Statistical analysis

This meta-analysis was performed using the online software Med Calc (https://metaanalysis.com/?gad_source=1&gclid=CjwKCAjwpsBhAiEiwALwsVYfV_ad8v_1RmprKd2OLar30QYPJFQSqehl9HHuLmxYVnpPgYclzFhoCb9wQAvD_BwE). The mean difference (MD) was calculated for studies that used exactly the same methods and measurement units for homocysteine as a CVD biomarker. We also compared the standardized mean difference (SMD) for selected studies that used different methods and units of measurement for similar outcomes. Of these, 15 studies were included in the meta-analysis. Data analyzes were conducted with I2 statistics to detect heterogeneity and meta-regression analysis was done to find the source of heterogeneity. When I2 values were <50%, we used the fixed effects model; otherwise, the random effects model was used.

RESULTS

The studies analyzed consisted of 11 cross-sectional studies (Ijaz, et al., 2015, Karim, et al., 2015, Prajapati, et al., 2015, Islam, et al., 2016, Kaur, et al., 2016, Chaudhary , et al., 2017, Qin, et al., 2017, Li, et al., 2021, Sun, et al., 2021, Teng, et al., 2022), 3 cohort studies (Pac-Kozuchowska, et al., 2018, Raffield, et al., 2018, Monasso, et al., 2021), 3 randomized controlled trials (Cerbone, et al., 2016, Fruzzetti, et al., 2016, Momeni1comma, et al., 2019) and 18 case-control studies (Islam, et al., 2015, Jain, et al., 2015, Kouzehgaran, et al., 2015, Ramkaran, et al., 2015, Iqbal, et al., 2016, İşgüven, et al., 2016, Lai, et al., 2017, Rallidis, et al., 2017, Gupta, et al., 2018, Vishwajeet, et al., 2018, Nedelcu, et al., 2021, Rafi, et al. ., 2021). Most studies found a positive association between homocysteine levels and carotid intima-media thickness (IMT-C). Homocysteine levels were higher in younger individuals (<40 years) and correlated with increased IMT-C. Lifestyle factors such as obesity and

smoking also influence homocysteine levels. The most investigated gene in relation to homocysteine was the Methylenetetrahydrofolate reductase (MTHFR) gene and its variants. Other genes were evaluated, but only mutations in the MTHFR gene showed significant changes in homocysteine levels. Homocysteine levels decreased with the use of medications such as oral contraceptives, L-thyroxine and antidiabetics.

The descriptive data of the studies collected are presented in Table I, according to gender, countries, interventional procedures, observational findings and association with homocysteine. Of the 33 studies that showed a significant association of homocysteine with outcomes, 15 studies (Islam, et al., 2015, Jain, et al., 2015, Karim, et al., 2015, Iqbal, et al., 2016, Islam, et al., 2016, Lai, et al., 2017, Qin, et al., 2017, Rallidis, et al., 2017, Shah, et al., 2018, Gupta, et al., 2018, Pac-Kozuchowska, et al., 2018, Raffield, et al., 2018, Teng, et al., 2022) were selected for the meta-analysis with similar outcomes. Figure 2 shows the geographic distribution of studies around the world. Figure 3 shows the meta-analysis with full fixed-effects models and random-effects models. The effect size was estimated from the DMP values. Heterogeneity tests indicated an I2 value of 97.98% with confidence intervals for I2 of 97.44-98.41 ($p<0.001$).

Figure 2 : Geographic Distribution

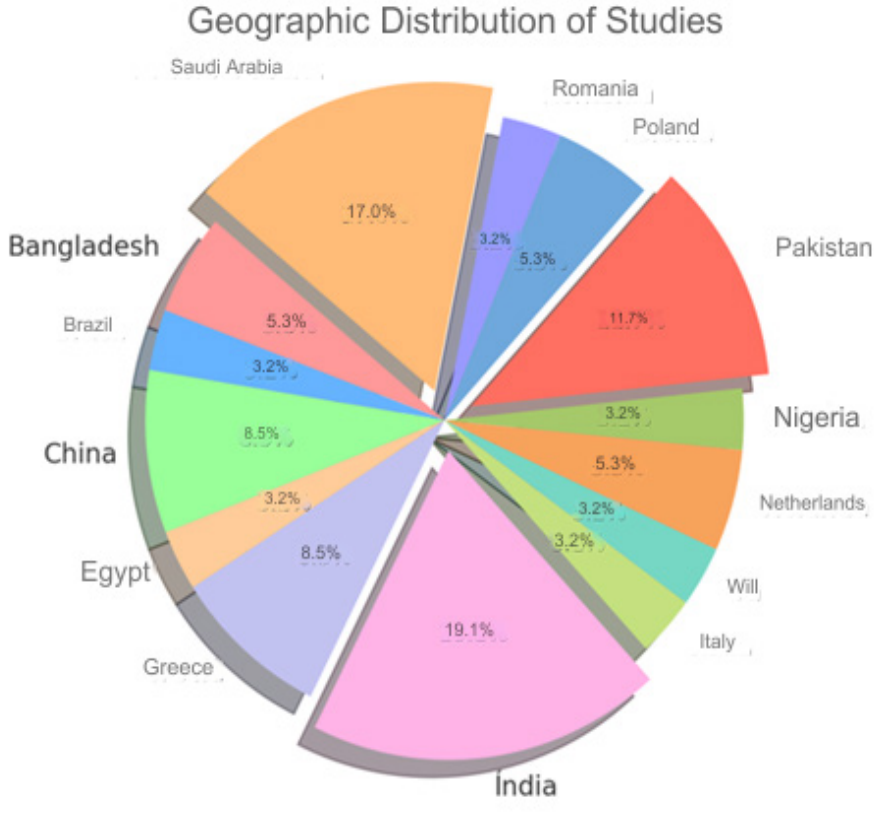


Table 1 Descriptive data of the studies collected

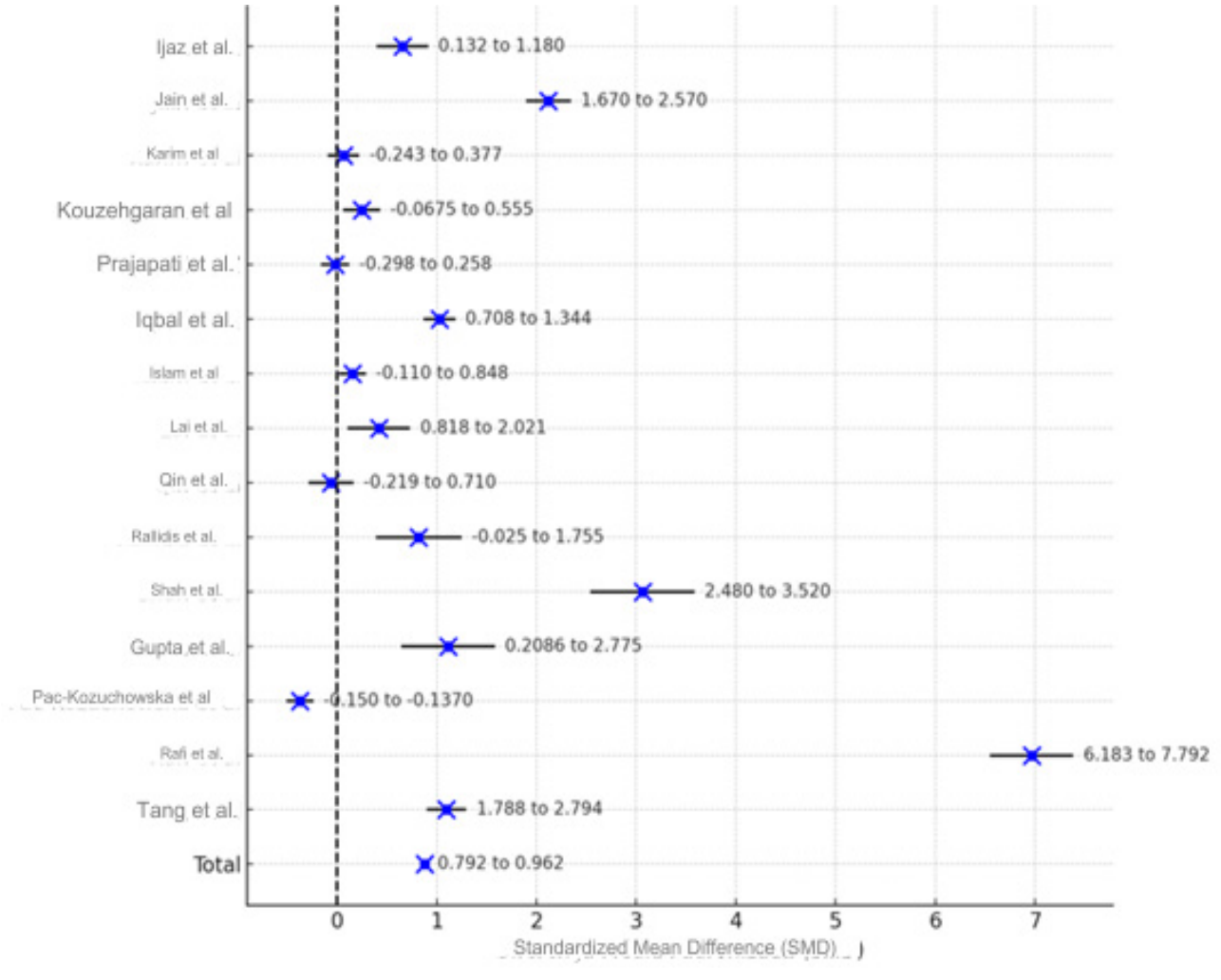
Study Characteristics	Number
Only Grown Men	1
Adult Women Only	7
Mixed Adults	23
Pediatric	7

Interventional Procedures	
Levothyroxine	1
Metformin	1
Oral contraceptive	1
Observational Findings	
Renal	3
Rheumatoid	two
Female Reproductive	two
Gastrointestinal	1
Micronutrients	3
Diet and Lifestyle	5
Genetic	5
Others	14
Findings of Association with Homocysteine	
Significant association of homocysteine with outcomes	33
Non-significant association of homocysteine with outcomes	5

DISCUSSION

Homocysteine has a high correlation with atherosclerotic cardiovascular disease (ASCVD) in young and overweight patients. Furthermore, the relationship of homocysteine with smoking, genetic polymorphism, specific hormonal and kidney disorders, nutritional deficiencies (vitamin B12 and folic acid) and the use of specific medications are among the other recurring findings. The data extracted from the compiled studies and the results produced in this systematic review provided a sufficient summary of the current literature related to homocysteine levels associated with premature cardiovascular disease.

Figure 3 : Forest plots of studies selected for meta-analysis with homocysteine as a biomarker for CVD



Genetic Role

From the studies collected, it was observed that homocysteine levels are influenced by several genetic associations. A randomized clinical trial was conducted to determine the influence of the MTHFR gene on the size of carotid intima-media thickness (IMT-C) in female patients with rheumatoid arthritis (Marini, et al., 2008). This gene is located on chromosome 1, at locus 1p36.3, and acts as a catalyst in the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.

The latter acts as a methyl group donor in the synthesis of methionine from homocysteine.

The results showed a significant increase in both IMT-C and homocysteine levels in 280 female patients. This raises the question whether people with MTHFR polymorphism are naturally predisposed to higher levels of homocysteine and, consequently, carotid intima-media thickness. This depends on the nature of the enzyme variant and its prevalence. For example, folate-correctable variants can be counterbalanced by increasing intake of folic acid supplements (Agodi, et al., 2011).

An investigation into the prevalence of MTHFR variants in the population of Tamil Nadu in southern India showed that MTHFR A1298C was more widespread than MTHFR C677T. It should be noted that of the 72 Tamilians tested, 52 had acute myocardial infarction (AMI), suggesting that the first variant may be more involved in the pathogenesis of CVD (Abd El-Aziz, Mohamed, 2017). Just having a gene polymorphism alone is not enough to guarantee premature CVD, as other factors need to be considered in addition to an increase in homocysteine.

To illustrate this, the same gene was studied again in a different study (Iqbal, et al., 2016) that analyzed a population of Pakistani patients with AMI. In addition to MTHFR C677T and MTHFR A1298C, polymorphisms of methionine synthase and cystathionine-beta-synthase were also considered. The research results showed that both patients with AMI and healthy controls (both with MTHFR polymorphism) had elevated homocysteine levels (23±17.2 and 23±13.4 mmol/l, respectively) above the normal limit (15 mmol /l), while the other two genes did not significantly alter homocysteine levels.

Despite these findings, there was no significant association of the MTHFR polymorphism with an increased risk of premature myocardial infarction in the Pakistani population (Iqbal, et al., 2016). Another study (Gupta, et al., 2018) examined how the Apolipoprotein (ApoE) polymorphism, along with other biochemical risk factors, such as homocysteine, would be associated with very young patients presenting with AMI.

Both ApoE and homocysteine were not significantly altered in these young patients, while other factors such as ApoA1 and HCL-C were significantly reduced, but only compared to healthy

controls and not to older patients presenting with acute AMI (Gupta, et al., 2018). From these results, it can be seen that elevated homocysteine is not an absolute outcome, even with polymorphism in related genes.

Essential Micronutrients

In addition to genes, nutrients play a substantial role in influencing homocysteine levels and IMT-C sizes. One study (Celik, Celik; 2018) examined the relationship between vitamin B12 levels and EMI-C size. All patients with vitamin B12 deficiency had not only higher EMI-C but also elevated homocysteine levels. This may be attributed to decreased autonomic function. As B12 is essential for the maintenance of nerve function, a deficiency would be expected to impair sympathetic and parasympathetic activity, which in turn affects the cardiovascular system (Celik, Celik; 2018).

Another study (Monasso, et al., 2021) also emphasized how deficiency of circulating vitamin B12 during fetal life can affect EMI-C in school-age children. This was a prospective cohort study that followed 3,826 children from early pregnancy to school age. Considering the normal levels of vitamin B12 and folate circulating during pregnancy (>145 pmol/L and >8 nmol/L, respectively), low levels of the former were associated with increased EMI-C, while low levels of the latter were associated with decrease in EMI-C.

Interestingly, homocysteine levels did not significantly relate to carotid intimal thickness, except in a standard deviation score, which showed that a high level in a blood sample taken from the umbilical cord was associated with lower IMT-C, but this was an exception (Monasso, et al., 2021). This may have been due to the extremely young age of the sample or the suppressive effect of vitamin B12 on homocysteine, which is why the effects of the latter were masked by the former. This is exemplified in another study, which showed how homocysteine was inversely proportional to both vitamin B12 and folic acid. Furthermore, homocysteine increased with age and would not be significantly high in children (Henry, et al., 2012).

Thyroid Hormone

Hormones can also interact with homocysteine. The effects of thyroxine on homocysteine were investigated in one study (Cerbone, et al., 2016); a total of 39 children with subclinical hypothyroidism were treated with L-thyroxine for more than 2 years, and several parameters were compared before and after the intervention. Weight-to-height ratio, triglyceride levels, atherogenic index, and homocysteine decreased significantly after therapy, while high-density lipoprotein (HDL-C) levels increased. Although the underlying reason has not been clarified, a separate study (İşgüven, et al., 2016) sought to determine the effects of thyroid autoimmunity (TA) in euthyroid girls diagnosed with Hashimoto's thyroiditis. The outcome was a measure of IMT-C and several other CVD risk factors, including homocysteine.

Here, the findings are contradictory to the previously mentioned study (Cerbone, et al., 2016). When comparing the diseased and control groups, there was not much difference in the following parameters: thyroid hormone levels, insulin levels, homocysteine levels and Homeostasis Model Assessment for Insulin Resistance (HOMA-IR). However, regardless of thyroid function, all patients showed increased IMT-C compared to the control group. It was concluded that TA was more related to chronic inflammation that caused endothelial dysfunction and not to the elevation of any specific marker of cardiovascular risk (İşgüven, et al., 2016). It appears that only particular hormonal diseases are associated with homocysteine, but this needs further investigation.

Female Reproductive System

Polycystic ovary syndrome (PCOS) is another hormonal disease known to be associated with vascular changes, such as increased intima-media thickness, increased arterial stiffness and endothelial dysfunction. This is also reflected by the elevation of certain surrogate markers of cardiovascular risk,

of which homocysteine is one of the main factors. An intervention to examine how administering metformin tablets (under the trade name Formaet, manufactured by Mylan MV Canonsburg, USA) to patients with PCOS would affect cardiovascular risk factors. The drug was administered at a dose of 850 mg per day for 6 months. Metformin significantly reduced only insulin, blood pressure, high-sensitivity C-reactive protein (Hs-CRP) and plasminogen activator inhibitor-1 levels. On the other hand, it can lead to elevated homocysteine levels, but to a lesser extent. Because many other risk factors have been suppressed, a slight increase in homocysteine should not be considered to place the patient at greater risk for cardiovascular events (20). But if metformin could indirectly affect homocysteine, can the same be said about other antidiabetic medications? One paper found that rosiglitazone (GSK plc, Brentford, UK) had a suppressive effect on homocysteine, and another found that sulfonylureas did not significantly alter homocysteine (Sullivan, et al., 2011). Whether these medications are preferred to metformin in CVD prevention is a question beyond the scope of this systematic review.

Endogenous female hormones are known to alter homocysteine levels. Therefore, it is expected that oral contraceptive pills may also influence homocysteine and lipid levels and indirectly affect CVD risk, but the literature presents contradictory results, so it has not been confirmed which finding is more valid. A study (Momeni1comma, et al., 2019) conducted in Iran compared the use of oral contraceptives (OC) among 100 women with normal menstrual cycles over a period of 3-6 months. In the group that used OCs for at least 24 to 36 months, higher levels of homocysteine, low-density lipoprotein (LDL), cholesterol, triglycerides, and systolic blood pressure were recorded. There was a significant difference between this group and the other groups according to the Tukey test, especially for homocysteine (Momeni1comma, et al., 2019). This should in no way be taken as evidence for the active use of OCPs as part of therapy for patients with CVD. Instead, it should be based on clinical judgment and other factors related to the patient's health status.

Kidney Pathologies

One study (Do Val., et al., 2019) sought to evaluate the association between left ventricular mass z-score and IMT-C with other risk factors. This study particularly looked at children and adolescents with end-stage kidney disease and compared them with healthy controls. Multivariate analysis revealed that left ventricular mass z-score was related to age, duration of dialysis, systolic blood pressure, serum hemoglobin levels, and HDL levels, while IMT-C was related to systolic blood pressure.

It is unclear why homocysteine was not significantly related to the aforementioned outcomes, especially considering that patients with chronic kidney disease typically present with hyperhomocysteinemia (Do Val., et al., 2019).

These findings were reinforced by another study (Aksu, et al., 2019), in which kidney disease in the sampled population was nephrotic syndrome. As it was already established that serum asymmetric dimethylarginine (ADMA) may be an independent risk factor for CVD (due to its ability to inhibit nitric oxide production), the study attempted to find any significant link between it and atherosclerotic risk factors. in children. As in the previous study (Do Val., et al., 2019), homocysteine was not found to be different between groups, nor was it associated with ADMA or EMI-C.

It can be deduced that kidney pathologies may interfere with the expected findings, but this needs to be elucidated in future studies (Aksu, et al., 2019). One kidney disease that has been found to correlate with elevated homocysteine levels is autosomal dominant polycystic kidney disease (ADPKD). It is a known fact that ADPKD can cause increased cardiovascular mortality, but the literature has not clarified the exact underlying pathogenesis, which is why a study (Lai, et al., 2017) was conducted to identify early and non-invasive markers of CVD in patients with ADPKD.

Study results revealed elevated homocysteine levels in addition to HOMA-IR, serum uric acid, renal resistance index, and left ventricular mass index. However, there was no significant increase in EMI-C (Lai, et al., 2017). Just as only certain hormonal diseases affect homocysteine or CVD risk

or both, the same appears to be observed in kidney diseases. This actually suggests that the dynamics involved between homocysteine, CVD and other risk factors/diseases may be more complex than estimated.

Age Factor

By analyzing the various risk factors including homocysteine and coronary plaque morphology by comparing young and elderly Indian patients with coronary artery disease below and above 40 years. Using computed tomography angiography, it was found that young patients had more pronounced features of positive remodeling, punctate calcification, and noncalcified plaques compared with older patients.

In addition, all patients with stable coronary angina had only a single vessel involved, whereas patients with acute coronary syndrome had multiple vessel involvement. The most commonly involved area was the proximal segment of the left anterior descending artery.

All young patients with acute coronary syndrome (ACS) had homocysteine levels greater than 15 μmol/L, but the difference between the two groups was not significant (Chaudhary, et al., 2017). This pattern of young patients being more predisposed to CVD due to hyperhomocysteinemia is evident throughout the literature collected. A leading lipoprotein factor related to premature CVD is lipoprotein(a), which is predominantly genetically inherited. In addition to the pro-inflammatory state, it is also related to the presence and severity of CVD (Habib, et al., 2013).

Gastrointestinal and Food Related

Among the diseases that predispose a patient, these seem to follow random patterns. But perhaps this simply reflects the paucity of knowledge we have about homocysteine itself, and this forms an incentive for even more in-depth research aimed at elucidating the intricacies of the amino

acid in the human body.

For the intima-media thickness of the superior carotid, the one that surrounds the gastrointestinal tract, ulcerative colitis (UC) is a significant example. Findings from a study (Jain, et al., 2015) on 60 patients with UC showed that not only IMT-C was significantly increased, but also homocysteine, HOMA-IR and insulin were significantly higher ($p<0$ .05). Furthermore, a significant correlation was observed between EMI-C and homocysteine, homocysteine and HOMA-IR levels (Jain, et al., 2015).

CONCLUSION

This systematic review study synthesized the evidence available in the literature on the relationship between serum homocysteine levels and the risk of early cardiovascular disease. Homocysteine is an intermediate in methionine metabolism, which can exert atherogenic, thrombogenic, hypertensive, cardiotoxic and neurotoxic effects, depending on its concentration and associated pathophysiological conditions.

Hyperhomocysteinemia is determined by genetic factors, such as the polymorphism of the enzyme methylenetetrahydrofolate reductase (MTHFR), and by environmental factors, such as smoking, obesity, diabetes, dyslipidemia, B vitamin deficiency, hypothyroidism, chronic renal failure and some inflammatory and autoimmune diseases.

Carotid intima-media thickness (IMT-C) is a marker of subclinical atherosclerosis, which reflects vascular damage and the risk of cardiovascular events, such as myocardial infarction and stroke. IMT-C is correlated with homocysteine levels and other cardiovascular risk factors and can be reduced by pharmacological and non-pharmacological interventions that modulate homocysteine metabolism.

Therefore, the assessment of IMT-C and homocysteine can contribute to the diagnosis, prevention, treatment and prognosis of cardiovascular diseases in young and adult individuals, healthy

or with comorbidities that affect homocysteine homeostasis and vascular function.

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