

Homocysteine as a predictor and prognostic marker of atherosclerotic cardiovascular disease: a systematic review and meta-analysis

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Abstract. – OBJECTIVE: Hyperhomocysteinemia is a well-known marker that is associated with an increased risk of atherosclerosis due to its toxic effect on endothelial cells. This, in turn, leads to cardiovascular injury and increases morbidity. Different studies have shown alterations in the levels of homocysteine with respect to multiple disease states. Whether this non-traditional marker is associated with cardiovascular injury or not is subject to conflicting results. The purpose of this systematic review is to evaluate the role of homocysteine in the formation of atherosclerotic cardiovascular disease in young adults and children.

MATERIALS AND METHODS: This systematic review was conducted following Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (PRISMA). A search was done using specific keywords, including “homocysteine”, “coronary artery disease”, and “atherosclerosis”, amongst several others, from the databases of PubMed, COCHRANE, and EBS-CO. The data items included the diseased sample population along with the intervention used, or investigations carried out and the findings of the studies. Finally, 35 eligible studies were included.

RESULTS: Young patients with atherosclerotic cardiovascular disease were more likely to have elevated levels of homocysteine compared to elderly patients. Elevated levels of homocysteine have been observed with several genetic, nutritional deficiencies, and autoimmune states such as rheumatoid arthritis. On the other hand, decreased levels of homocysteine have been observed after certain intervention treatments, such as oral contraceptive pills, L-thyroxine, and even the adoption of certain diets. In the majority of studies, whenever homocysteine levels were higher than normal, this was reflected by an increased carotid intima-media thickness.

CONCLUSIONS: Homocysteine has a high correlation with atherosclerotic cardiovascular disease in young and overweight patients. In addition, the relationship of homocysteine with smoking, genetic polymorphism, specific hormonal and renal disorders, nutritional deficiencies

(vitamin B12 and folic acid), and the use of specific medicines are among the other recurring findings. Given that many of these studies focus only on women, the relationship between homocysteine and atherosclerotic cardiovascular diseases in males is still unclear. Whether males are more prone to hyperhomocysteinemia needs to be assessed. Still, precise processes underlying variations in homocysteine in relation to all influencing factors are unclear and need further studies.

Key Words:

Coronary artery disease, Vascular disease, Atherosclerosis, Homocysteine.

Introduction

Among the numerous markers that have been studied in the literature to determine the likelihood of cardiovascular disease (CVD)¹⁻⁴, homocysteine is considered to be one of the promising independent, non-traditional and debatable risk factors for coronary artery disease⁵. The biological substance homocysteine itself is actually an amino acid that is formed by the conversion of methionine to cysteine⁶. More severe elevations can be seen in patients with defects or mutations of the metabolic enzymes involved in the conversion process, which include cystathionine β -synthase and 5,10-methylene tetrahydrofolate reductase (MTHFR)⁶. Elevations of homocysteine are seen in individuals with deficiencies of vitamin B12 or folic acid, but these alterations are modest⁷. Other scenarios that can result in hyperhomocysteinemia include methotrexate overdose/toxicity or in patients with impaired liver or renal function⁸.

The main concern regarding high levels of circulating homocysteine is its impact on endo-

thelial cells lining vessels. It also interferes with low-density lipoprotein oxidation and has pro-thrombotic properties⁵. In addition, many patients with CVD have high levels of non-traditional risk factors, including homocysteine⁹. The burden of disease in younger individuals with atherosclerotic cardiovascular diseases (ASCVD) is a rising problem globally^{10,11}. Due to the possible loss of lifetime productivity and greater lifetime health-care expenses, the burden of ASCVD in young adults is a significant public health issue.

Well-known facts that increase the risk of ASCVD include smoking, diabetes, hyperlipidemia, hypertension, metabolic syndrome, prothrombotic conditions, and a proinflammatory state⁸. Smoking raises the risk of peripheral arterial disease and abdominal aortic aneurysm by five times, as well as the risks of CVD, stroke, and sudden cardiac death by two and three times, respectively⁹. In fact, it is the presence of multiple confounding factors that have led to conflicting findings with respect to homocysteine levels and CVD. The purpose of this systematic review is to compile all the existing literature on homocysteine and its effect on ASCVD in young adults and children. These results will help to determine which disease states, patient characteristics, drugs/interventions, and markers affect homocysteine levels and, indirectly, the risk for CVD. This can then be used clinically to decide the appropriate management based on the homocysteine levels recorded from blood investigations.

Materials and Methods

Search Strategy

This review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Figure 1). The time frame of articles selections ranged from January 2015 to September 2022. The databases searched included PubMed, COCHRANE, and EBSCO. The search strategy involved using specific keywords in combination with the conjunctions “OR” and “AND”. These keywords included “coronary artery disease”, “coronary disease”, “coronary heart disease”, “vascular disease”, “atherosclerosis”, “arteriosclerosis”, and “homocysteine “. In the second phase, two separate reviewers examined the search results; initially, they looked at the titles and abstracts and omitted any studies that were not relevant. Abstracts that were related to our

subject matter were considered for a full review. The entire texts of all pertinent articles and those that required more thorough study were then obtained and checked for eligibility once again.

A total of 2,596 studies were screened. After pre-screening was done and removal of 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. In this phase, about 1,983 studies were excluded. All 41 remaining studies were carefully reviewed for inclusion criteria and there were 6 studies that did not fulfill the inclusion criteria. Finally, about 35 studies were included in the final systematic review analysis. The details are mentioned in Figure 1, which shows the flow diagram of the whole article selection process according to the PRISMA guidelines.

Inclusion and Exclusion Criteria

The inclusion criteria were studies that included homocysteine as a cause of premature myocardial infarction or coronary artery disease for patients below the age of 45, regardless of gender. Case-control, cohort, and cross-sectional types of study of human subjects published in English were included. Exclusion criteria included studies published before January 2015 and after September 2022. Conference abstracts, review articles, research thesis, editorials, commentaries, opinions, viewpoints, case reports, and systematic reviews were all excluded. Duplicates and retracted articles that did not fulfill the inclusion criteria were automatically filtered out.

Data Review and Extraction

The databases PubMed, COCHRANE, and EBSCO were searched, and the keywords were entered. All results were then exported to Endnote 20.0, where the titles and abstracts were reviewed. Data collected was then compiled into tables. Two independent authors served as study reviewers and extracted the following data items: authors, type of study, intervention group, control/comparison group, measurable outcomes, and findings.

Statistical Analysis

This meta-analysis was performed using the online Med Calc software (<https://www.medcalc.org/manual/meta-analysis.php>). We calculated the mean difference (MD) for studies that used precisely the same measurement methods and

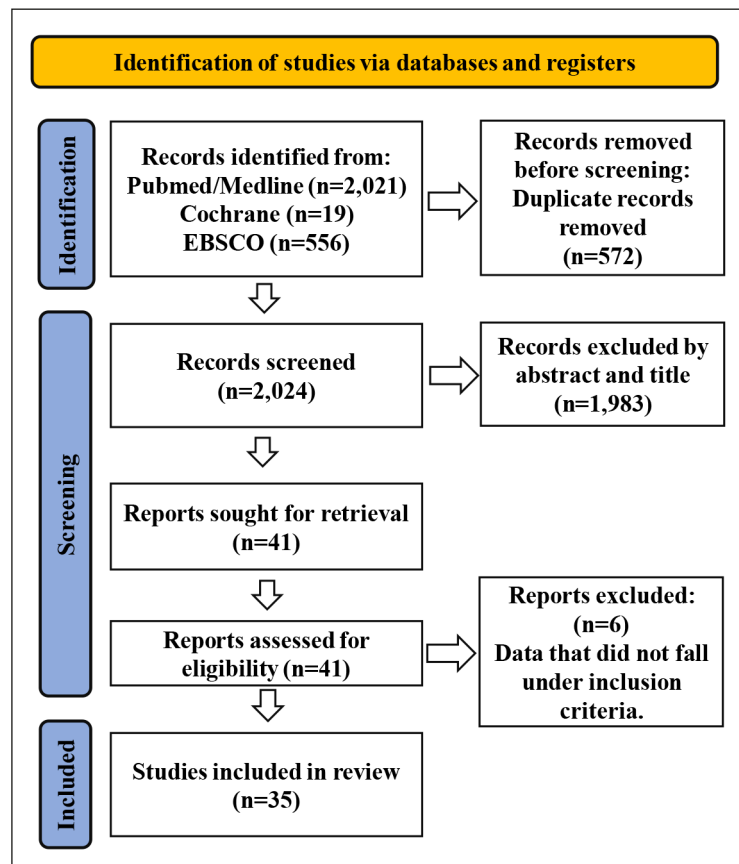


Figure 1. Flow diagram of article selection according to the PRISMA guidelines.

units for Homocysteine as biomarker of CVD. We also compared the standard mean difference (SMD) for the studies selected that used different measurement methods and units for similar outcomes. About 15 studies were included in the meta-analysis. Data analyses were conducted with I^2 statistics to detect heterogeneity and meta-regression analysis was done to find the source of heterogeneity. When I^2 values were $<50\%$, we would use the fixed-effects model; otherwise, the random-effects model was used. All data were analyzed with 95% CI. The results were plotted in the form of forest plots, with p -values lower than 0.05 considered significant.

Results

There were 11 cross-sectional studies^{12,15,17,23,24,26,27,42,44-46}, 3 cohorts^{33,34,40}, 3 randomized control trials^{19,20,39}, and the remaining were case-control studies^{13,14,16,18,21,22,25,28-32,35-38,41,43}. This, along with all other descriptive characteristics ex-

tracted from the studies¹²⁻⁴⁶ are included in **Supplementary Table I**. From the literature review in **Supplementary Table I**, several recurring points were noted. In most investigations, elevated levels of homocysteine were always associated with an increase in carotid intima-media thickness (CIMT). Younger people (<40) generally had higher levels of homocysteine and were more predisposed to increased size of CIMT. Along with this, lifestyle factors such as obesity and smoking played roles in elevated homocysteine. The most studied gene that was related to homocysteine was the *Methylenetetrahydrofolate reductase* (*MTHFR*) gene and its variants. While a number of other genes were also analyzed, only defects of the *MTHFR* gene produced significant changes in homocysteine. Homocysteine levels have been reported to drop when people use medications like oral contraceptives, L-thyroxine, and anti-diabetic drugs.

Table I shows the descriptive data of collected studies according to gender, countries, interventional procedure, observational findings, and ho-

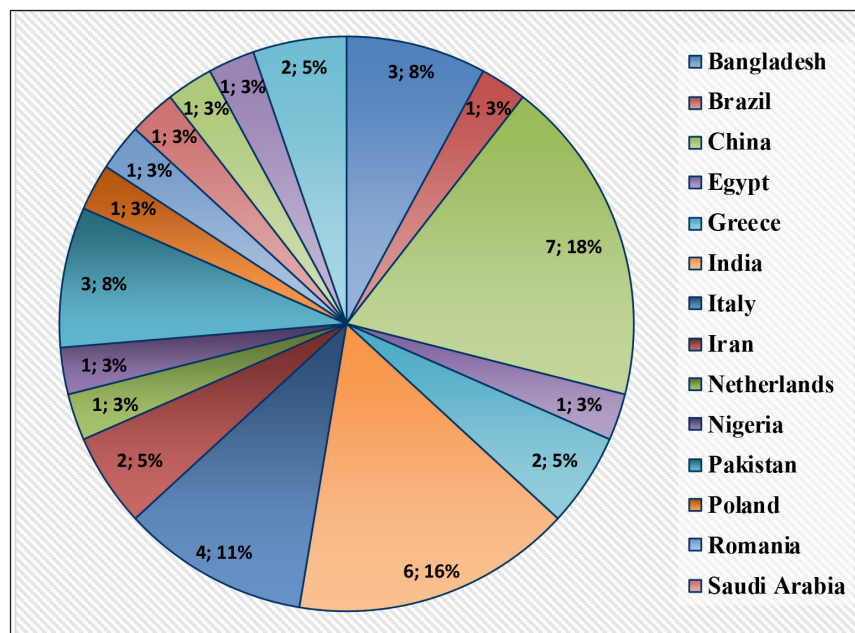


Figure 2. Geographical distribution of studies across the globe (values; percentages).

homocysteine association findings. The table reveals 33 studies¹²⁻⁴⁶ with significant association of homocysteine with outcomes. Figure 2 shows the geographical distribution of studies across the globe.

Figure 3 shows the meta-analysis of 15 studies^{13,14-17,21,23,25,27-29,32,33,34,46} that were selected with

similar outcomes. Both total fixed effects and random fixed effects models were computed. The effect size was calculated from SMD values. Tests of heterogeneity showed I^2 value of 97.98% with confidence limits for I^2 97.44-98.41 ($p < 0.001$).

Table I. Descriptive data of the collected studies.

Feature of study	Number
Adult Male only	1
Adult Female only	7
Adult Mixed	23
Pediatric	7
Interventional procedure	
Levothyroxine	1
Metformin	1
Oral contraceptive	1
Observational findings	
Renal	3
Rheumatoid	2
Female reproductive	2
Gastrointestinal	1
Micronutrients	3
Diet and Lifestyle	5
Genetic	5
Other	14
Homocysteine association findings	
Significant association of homocysteine with outcomes	33
Non-significant association of homocysteine with outcomes	5

Discussion

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

Genetic Role

From the collected studies, it was observed that homocysteine levels are influenced by a number of genetic associations. A randomized control trial⁴⁷ was conducted to determine the influence of the *MTHFR* gene on CIMT size in female patients with rheumatoid arthritis. This is a gene that is found on chromosome 1 at the locus of 1p36.3, and functions as a catalyst for the reduction (irreversible) of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The latter acts as a donor for the methyl group in the synthesis of methionine from homocysteine. The results showed a significant increase in both CIMT and homocysteine levels in 280 female patients. This arises the question regarding whether people with poly-

morphism of *MTHFR* are naturally predisposed to higher homocysteine and in turn CIMT. This depends on the nature of the enzyme variant and its prevalence. For example, folate-remedial variants can be countered by increasing the intake of folic acid supplementation⁴⁸. An investigation of the prevalence of *MTHFR* variants in the South Indian Tamil Nadu population showed that *MTHFR A1298C* was more widespread than *MTHFR C677T*. It should be noted that of the 72 Tamilians tested, 52 had an acute myocardial infarction (AMI) hinting that the former variant may be more involved in the pathogenesis of CVD⁴⁹. Just having a polymorphism of the gene on its own is not enough to ensure premature CVD, as other factors need to be considered besides a rise in homocysteine alone. To illustrate this, the same gene was studied once again in a different research²¹ that looked at a population of Pakistani patients with AMI. Alongside *MTHFR C677T* and *MTHFR A1298C*, methionine synthase and cystathionine-beta-synthase polymorphism were also considered. The results of the research showed how 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 alter homocysteine levels greatly. Despite these findings, there was no significant association of the *MTHFR* polymorphism with an increased risk

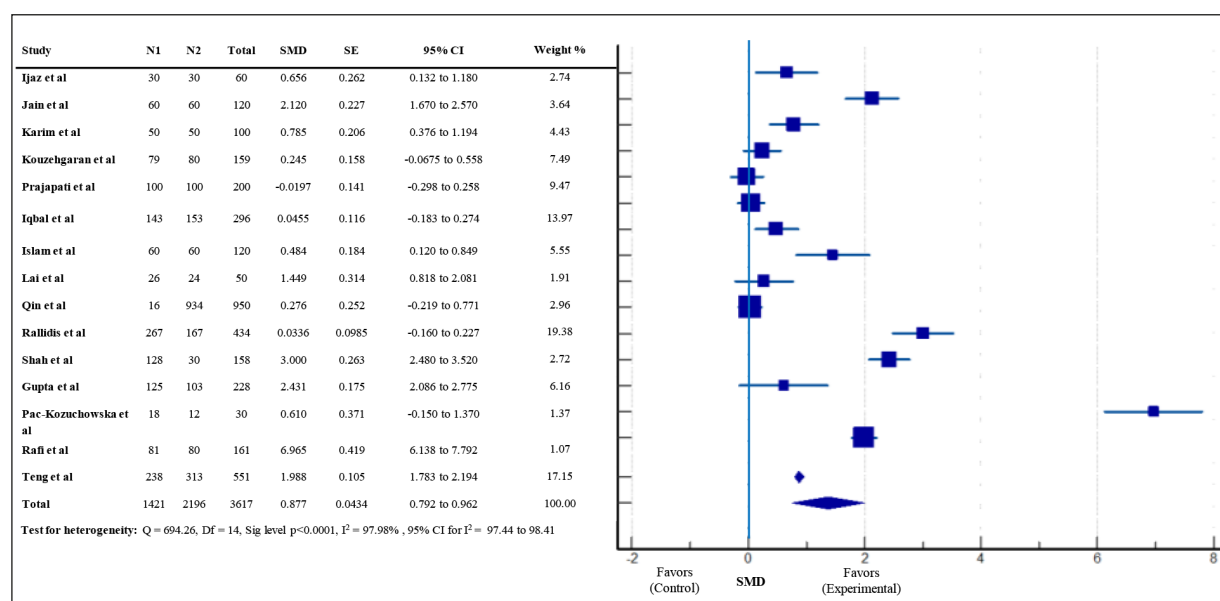


Figure 3. Forest plots of studies selected for meta-analysis with homocysteine as a biomarker for CVD.

of premature myocardial infarction in the Pakistani population²¹. Another study³² examined how 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 lowered, but only in comparison to healthy controls and not older patients presenting with acute MI³². From the aforementioned, we can see that elevated homocysteine is not an absolute outcome, even with polymorphism of the related genes.

Essential Micronutrients

Besides genes, nutrients play an essential role in influencing homocysteine levels and CIMENT sizes. One study³⁰ examined the link between vitamin B12 levels and CIMENT size. All patients with vitamin B12 deficiency showed not only higher CIMENT but also elevated homocysteine levels. The reason for this can be attributed to decreased autonomic function. Since B12 is essential for the maintenance of nervous function, a deficiency would be expected to impair sympathetic and parasympathetic activity, which in turn affects the cardiovascular system³⁰. One other study⁴⁰ also emphasized how a deficiency of circulating vitamin B12 during fetal life can affect CIMENT in school-age children. This was a prospective cohort study that looked at 3,826 children from early pregnancy till their school age. When considering normal circulating levels of vitamin B12 and folate during pregnancy (>145 pmol/L and >8 nmol/L, respectively), low levels of the former were associated with increased CIMENT, while low levels of the latter were associated with decreased CIMENT. Interestingly, homocysteine levels did not significantly relate to carotid intima thickness except in one standard deviation score, which showed that the high level in a blood sample taken from the umbilical cord was associated with lower CIMENT, but this was an exception⁴⁰. It may have been due to the extremely young age of the sample or because of the suppression 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. Moreover, homocysteine increased with age and would not be significantly high in children⁵⁰.

Thyroid Hormone

Hormones can also interact with homocysteine. The effects of thyroxine on homocysteine were investigated in a study¹⁹; a total of 39 children with subclinical hypothyroidism were treated with L-thyroxine for over 2 years, and the various parameters were compared before and after the intervention. Weight-to-height ratio, triglyceride levels, atherogenic index, and homocysteine had significantly decreased following therapy, while high density lipoprotein (HDL-C) levels became higher. While the underlying reason has not been clarified, a separate study²² sought to determine the effects of Thyroid Autoimmunity (TA) in euthyroid girls diagnosed with Hashimoto's thyroiditis. The outcome was a measure of CIMENT and various other risk factors of CVD, including homocysteine. Here, the findings are contradictory to the previously mentioned study¹⁹. When comparing the diseased and control groups, there was not much difference in the following parameters: thyroid hormone levels, insulin levels, homocysteine levels, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). However, irrespective of thyroid function, all patients had increased CIMENT compared to the control group. It was concluded that TA was more related to chronic inflammation that caused endothelial dysfunction and not the elevation of any particular cardiovascular risk marker²². It would appear that only particular hormonal diseases are associated with homocysteine, but this needs further workup.

Female Reproductive System

Polycystic ovarian syndrome (PCOS) is another hormonal disease that has been known to be associated with vascular alterations such as increased intima-media thickness, increased arterial stiffness, and endothelial dysfunction. This is also reflected by the elevation of certain cardiovascular risk surrogate markers, of which homocysteine is one of the leading factors. An intervention to examine how the administration of metformin tablets (under the brand name Formet, manufactured by Mylan M.V. Canonsburg, USA) to patients of PCOS would affect the cardiovascular risk factors. The drug was given at a dose of 850 mg a day for 6 months. Metformin only significantly reduced insulin, blood pressure, high-sensitivity C-reactive protein (Hs-CRP) levels, and plasminogen activator inhibitor-1. Conversely, it might lead to elevated

homocysteine levels but to a minor extent. Since many other risk factors were suppressed, a mild rise in homocysteine should not be considered as putting the patient at greater risk for cardiovascular events²⁰. But if metformin could indirectly affect homocysteine, can the same be said about other anti-diabetic medications? One article found rosiglitazone (GSK plc, Brentford, UK) had a suppressive effect on homocysteine, and another found that sulphonylureas did not alter homocysteine significantly^{51,52}. Whether these medications are preferred over metformin in preventing CVD is a question that goes outside the scope of this systematic review.

Endogenous female hormones themselves are known to alter homocysteine levels. Therefore, it is expected that oral contraceptive pills can also influence homocysteine and lipid levels, and indirectly affect the risk for CVD, but the literature presents contradictory results, so it has not been confirmed which finding is more valid. One study³⁹ conducted in Iran compared oral contraceptive (OCP) use amongst 100 women with normal menstrual cycles for a period of 3-6 months. In the group that used OCPs for at least 24 to 36 months, higher levels of homocysteine, low-density lipoprotein (LDL), cholesterol, triglyceride, and systolic blood pressure were recorded. A significant difference existed between this group and the other groups according to Tukey's test, especially for homocysteine³⁹. This by no means should be taken as evidence to actively use OCP as part of therapy for patients with CVD. Instead, it should be based on clinical judgment and other factors pertaining to the health status of the patient.

Renal Pathologies

A study⁵³ sought to evaluate the association between left ventricular mass z score (LVMZ) and CIMT with other risk factors. This study particularly looked at children and adolescents with end-stage renal disease and compared them with healthy control. Multivariate analysis revealed that LVMZ was linked with only age, dialysis duration, systolic blood pressure, serum hemoglobin, and HDL levels, while CIMT was only linked with systolic blood pressure. It is not clear why homocysteine was not significantly related to the aforementioned outcomes, especially since patients with chronic kidney disease normally have hyperhomocysteinemia⁵³. These findings were reinforced by another study⁵⁴, except the renal disease in the sample population taken was nephrotic

syndrome. Since 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 tried to find any significant link between it and atherosclerotic risk factors in children. Much like the previous study⁵³, homocysteine was not found to be different between groups, nor was it associated with either ADMA or CIMT. One might deduce that renal pathologies may interfere with the expected findings, but this needs to be elucidated in further studies⁵⁴. One renal disease that was found to be correlated with elevated homocysteine levels is autosomal dominant polycystic kidney disease (ADPKD). It is a well-known fact that ADPKD might cause increased cardiovascular mortality, but the literature has not clarified the exact underlying pathogenesis, which is why a study²⁵ was conducted on identifying early and non-invasive markers of CVD in patients of ADPKD. The results of the study revealed elevated levels of homocysteine besides HOMA-IR, serum uric acid, renal resistive index, and left ventricular mass index. However, there was no significant increase in CIMT²⁵. Much like how only certain hormonal diseases affect homocysteine alone or risk of CVD alone or both, the same seems to be observed in renal diseases. This actually hints that the dynamics involved between homocysteine, CVD, and other risk factors/diseases may be more complex than estimated.

Age Factor

When analyzing the various risk factors, a study looked at several of them, including homocysteine and coronary plaque morphology, when comparing young and old Indian patients with coronary arterial disease below and above the age of 40 years. By using computed tomography angiography, it was found that young patients had more pronounced features of positive remodeling, spotty calcification, and non-calcified plaques compared to older patients. In addition, all patients with coronary stable angina were involved in only a single vessel, while patients with acute coronary syndrome had multi-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 of more than 15 $\mu\text{mol/L}$, but the difference between the two was not significant²⁶. This pattern of young patients being more predisposed to CVD due to hyperhomocysteinemia is something that is obvious from

all the literature collected. A leading lipoprotein factor related to premature CVD is lipoprotein(a), which has predominantly genetic inheritance⁵⁵. Moreover, the proinflammatory state is also related to the presence and severity of CVD⁵⁶.

Gastrointestinal and Food-Related

Amongst the diseases that predispose a patient to higher CIMT, one that involves the gastrointestinal tract, is ulcerative colitis (UC). Findings from a study¹⁴ on 60 patients with UC showed how not only CIMT was significantly raised, but also homocysteine, HOMA-IR, and insulin were significantly higher ($p<0.05$). Moreover, a significant correlation was observed between CIMT and homocysteine levels, homocysteine, and HOMA-IR¹⁴.

Amongst the various types of intervention used to lower homocysteine levels found in literature, specific types of diet have been proven to significantly lower homocysteine level. One article investigated the association of ACS in individuals who adopted the Mediterranean diet. An increase in homocysteine levels was associated with a 1% and 3% higher likelihood of ACS among younger (<45 years) and middle-aged (45-60 years) adults. Moreover, homocysteine was associated with 3% increase in the likelihood of ACS among those who did not adhere to the Mediterranean diet. Therefore, Mediterranean diet can be used as a prophylactic diet to prevent CVD³⁷.

Conclusions

The data extracted from the compiled studies and results produced in this systematic review provided a sufficient summary of the current literature relating to the homocysteine levels associated with premature cardiovascular disease. Homocysteine was highly correlated with atherosclerotic cardiovascular disease in young and overweight. In addition, other reproducible findings include an association of homocysteine with smoking, genetic polymorphism, certain hormonal and renal diseases, nutritional deficiencies (vitamin B12 and folic acid), and levothyroxine (which is under the trade name Levoxyl, Pfizer Inc, NY, USA). It is still not clear if males are more predisposed to hyperhomocysteinemia, as most of these studies^{20,22,27,33,39,43,45} were concentrated in females. What is even more ambiguous is the exact mechanism of changes in homocysteine with regard to all factors that influence, as

these seem to apparently follow random patterns. But perhaps this just reflects the dearth of knowledge we have on homocysteine itself, and this forms an incentive for even further research for the purpose of elucidating the intricacies of the amino acid in the human body.

Conflicts of Interest

The authors declare no conflict of interest.

Ethics Approval

Not applicable.

Informed Consent

Not applicable.

Availability of Data and Materials

All data and materials used and analyzed in the study can be obtained from the corresponding author on reasonable request.

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