Ghrelin and ghrelin/total cholesterol ratio as independent predictors for coronary artery disease: a systematic review and meta-analysis

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ABSTRACT

The present meta-analysis aimed to summarize the available data regarding the circulating levels of ghrelin in patients with cardiovascular diseases (CVDs). A comprehensive search was performed in electronic databases including PubMed, Scopus, EMBASE, and Web of Science up to January 20, 2021. Since the circulating levels of ghrelin were measured in different units across the included studies, they were expressed as the standardized mean difference (SMD) and 95% CI (summary effect size). A random-effects model comprising the DerSimonian and Laird method was used to pool SMDs. Sixteen articles (20 studies) comprised of 1087 cases and 437 controls were included. The pooled results showed that there were no significant differences between cases and controls in terms of ghrelin levels (SMD=−0.61, 95% CI −1.38 to 0.16; p=0.120; I^2=96.9%, p<0.001). The ghrelin concentrations in the CAD stratum were significantly lower than in controls, whereas they increased in other disease strata. New combined biomarkers demonstrated a significant decrease in the SMD of the ghrelin/total cholesterol (TC) ratio (−1.02; 95% CI −1.74 to −0.29, p=0.000; I^2=94.5%). However, no significant differences were found in the SMD of the ghrelin/high-density lipoprotein cholesterol ratio, ghrelin/low-density lipoprotein cholesterol ratio, and ghrelin/triglyceride (TG) ratio in cases with CVDs compared with the control group. Ghrelin was associated with CAD; therefore, it may be considered a biomarker for distinguishing between patients with and without CAD. Furthermore, the ghrelin/TC ratio could be proposed as a diagnostic marker for CVD.

INTRODUCTION

As the leading cause of high rates of morbidity and mortality worldwide, cardiovascular disease (CVD) refers to a combination of linked pathologies which include coronary heart disease (CHD), peripheral arterial disease, cerebrovascular disease, and venous thromboembolism, along with rheumatic and congenital heart diseases. The risk factors for CVD are dyslipidemia, smoking, hypertension, diabetes, and abdominal obesity.[1–3] As the prevalence of CVD risk factors rises, the global rate of CVD is expected to increase as well.[4] The WHO estimates that more than 75% of premature CVD cases are preventable and improving risk factors can help decrease the growing CVD burden.[2] As a critical chronic condition, obesity has been found to be linked with CVD.[5] White adipose tissue is a key player in obesity-mediated CVD. It is an active endocrine organ that secretes a variety of adipokines that affect the whole-body homeostasis via different signaling pathways and chemical mediators.[6] Ghrelin is an important adipokine, which plays a pivotal role in numerous processes in the body.[7 8] This factor is widely distributed in the heart and is related to cardiovascular risk factors. Ghrelin is implicated in atherosclerosis as the leading cause of coronary artery disease (CAD). Indeed, depending on the atherosclerosis stage, ghrelin has a dual modulatory function in the vascular system.[9] Thus, the evaluation of this adipokine in patients with CVD appears to be beneficial.[10–12]

Ghrelin is an adipocyte-derived orexigenic hormone and an endocrine regulatory peptide that serves multiple functions such as increasing appetite and regulating a number of biological cardiovascular processes.[8 13 14] Non-acylated ghrelin, which is the most abundant form of circulating ghrelin (80%–90%), has been demonstrated to exhibit growth hormone (GH)-releasing capacity. It also has several physiological functions in the cardiovascular system and lipid and glucose metabolism.[15] Moreover, significant associations between the levels of this adipokine and body mass index (BMI), high-density lipoprotein cholesterol (HDL-c) levels, diabetes, and fasting glucose have been reported.[16]

Ghrelin is predominantly generated in X/A-type cells of the gastrointestinal tract; however, its production has been observed in other tissues including the myocardium.[17 18] It has been reported that ghrelin and its receptors exist in the cells of the cardiovascular system, including cardiomyocytes and endothelial cells,[19–21] and this peptide has been shown to exhibit cardioprotective activities,

such as inhibiting endothelial apoptosis and inflammation, regulating blood pressure, serving as a vasodilator, and increasing the left ventricular function.[22–24] Thereby, due to its anti-inflammatory effects, and given that it is involved in inhibiting the activation of nuclear transcription factor kappa B (NF-κB) and the production of inflammatory cytokines in human endothelial cells, ghrelin plays a critical protective function against atherosclerosis and CVDs.[25 26] The evidence indicates that high levels of ghrelin in plasma could protect healthy individuals against CHD events or deaths.[27] Moreover, it was revealed that there is a negative correlation between ghrelin levels and the severity of CAD.[28 29] These findings confirm the prognostic value of serum ghrelin levels in patients with coronary atherosclerosis and CVDs.

Despite the existence of conventional risk prediction algorithms, the lack of appropriate CVD biomarkers is a critical issue.[30] Thus, early detection of risk factors is crucial for disease prevention and morbidity reduction.[31] There is accumulating evidence that suggests the involvement of ghrelin in cardiovascular function even, although its causative association with CVDs is still debated.[9] The present systematic review and meta-analysis aimed to summarize the available data regarding the circulating levels of ghrelin with possible atheroprotective functions in patients with CVDs.

METHODS

Search strategy

The meta-analysis was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines.[32] The English language studies published until January 20, 2021, were obtained from electronic databases, including PubMed/Medline, Scopus, EMBASE, and Web of Science. The search included the following MeSH (Medical Subject Heading) terms and relevant keywords: (Ghrelin OR "GHRL Protein" OR Ppghrelin OR "Motilin Related Peptide" OR "Motilin-Related Peptide" OR PpMTLRP OR Obestatin OR "Appetite-Regulating Hormone" OR "Appetite Regulating Hormone" OR "Gastric MLTRP") AND ("Heart Disease" OR "Heart" OR "Coronary Heart Disease" OR "Coronary Artery Disease" OR "CAD" OR "Artery Disease" OR "Coronary Arteriosclerosis" OR "Coronary Atherosclerosis" OR "Atherosclerosis" OR "Ischemia" OR "Myocardial" OR "MI" OR "Myocardial Ischemia" OR "Ischemic Heart Disease" OR "Heart Attack" OR "Heart Disease" OR "Ischemic" OR "Acute Coronary Syndrome" OR "Coronary Syndrome" OR "Acute Coronary" OR "ACS" OR "Angina" OR "Stable Angina" OR "Chronic Stable Angina" OR "Unstable Angina" OR "Coronary Disease" OR "Coronary" OR "Coronary Stenosis" OR "Stenosis" OR "Myocardial Infarction" OR "Infarction" OR "Cardiovascular Stroke" OR "Stroke" OR "Cardiovascular" OR "Myocardial Infarct" OR "Infarct" OR "Non-ST Elevated" "Myocardial Infarction" OR "Non ST Elevated Myocardial Infarction" OR "NSTEMI" OR "Non-ST-Elevation Myocardial Infarction" OR "Non-ST-Elevation Myocardial" OR "Non-ST-Elevation" OR "Non ST Elevation Myocardial Infarction" OR "ST Elevation Myocardial Infarction" OR "ST Segment Elevation Myocardial Infarction" OR "ST Elevated Myocardial Infarction" OR "STEMI" OR "Atherosclero*" OR "Cardio*" OR "Coronary Occlusion" OR "Stenosis"). In addition, the references of the relevant articles were manually checked for additional desired studies.

Study selection

Original studies of any design, including case-control, cross-sectional, and clinical cohort studies, studies providing detailed information regarding serum or plasma ghrelin levels in patients diagnosed with heart diseases such as CAD, myocardial infarction (MI), CHD, congestive heart failure, atrial fibrillation, heart failure, ischemic heart disease, and hypertensive heart disease and controls (participants without heart diseases and other chronic/metabolic conditions), and studies published in English met the inclusion criteria. Studies using animal models, tissue-based cultures, cell cultures (in vitro or ex vivo), and mRNA expression, as well as case reports, conference abstracts, comments or review articles, editorials, and articles without original data were excluded from the meta-analysis.

Two independent investigators (TL and FM) reviewed the title and the abstract of each article. Following this initial screening step, potential articles were included in our full-text review process. Any existing discrepancies were resolved by consensus or consultation with a third author (MZ).

Data extraction

Three individual authors (MA, AA, and SS) extracted the data using predesigned data collection sheets in Excel. The first author's name, year of publication, study design, sample size, geographical region, as well as age, BMI, comorbidities, type of heart disease, the levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), HDL-c, and ghrelin concentration in patients with CVD and controls (all means±SD) were recorded.

Quality assessment

The quality of the study was evaluated using the Newcastle-Ottawa Quality Assessment Scale (NOS), which involved the evaluation of study design and analysis, selection bias, measurements of exposure and outcome, and the generalizability of the results. The NOS tool includes nine items with scores ranging from 0 to 9. Based on the type of study, quality scores ≥5 in cross-sectional designs and scores ≥7 in case-control or cohort designs represented good quality.

Statistical analysis

All statistical analyses were conducted using STATA V.11.0 (STATA Corp, College Station, Texas, USA). Since the circulation levels of ghrelin were measured in different units across the included studies, they were expressed as the standardized mean difference (SMD) and 95% CI (summary effect size) using Hedges and Olkin SE. Moreover, with regard to the bias-correlation factor in effect size, an exact computation was used. A random-effects model with the DerSimonian and Laird method was used to pool SMDs. χ^2 and inconsistency index (I^2) statistics were also applied to assess inter-study heterogeneity.[33] To explore the source of heterogeneity, subgroup analyses were conducted on the continent where the study was performed and study design,

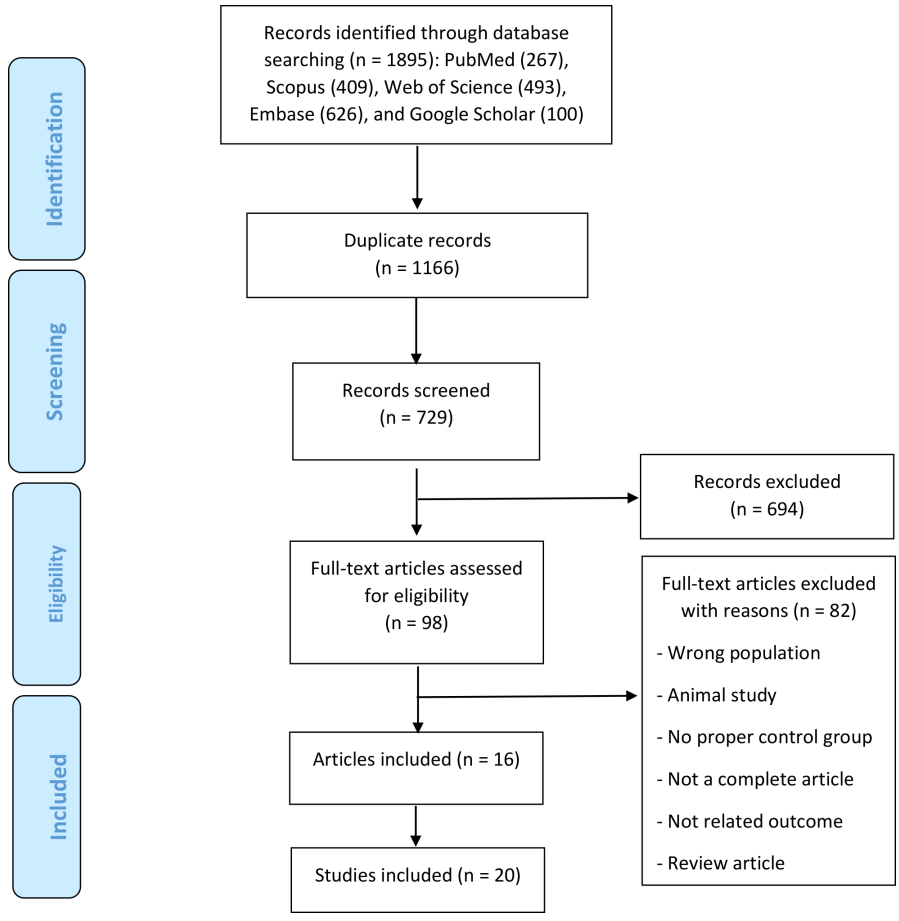

Figure 1 The flowchart of the study identification and selection process.

as well as the body fluid, type of disease, and other existing medical conditions of the participants. Meta-regression and sensitivity analyses were performed as additional assessments. Funnel plot and Egger's test were also used to examine the potential publication bias in our meta-analysis.

RESULTS

Literature search and study characteristics

The flowchart of the data selection process is shown in figure 1. The primary systematic search led to the retrieval of 1895 records, out of which 1166 were excluded as duplicates and 729 remained as the screened records, resulting in 16 articles (20 studies) as the final records for our meta-analysis.[16 34–48] The included studies, which were published from 2001 to 2021, were comprised of 1087 cases and 437 controls. Twenty studies reported data on CAD, while the remaining articles investigated other CVDs. Twelve studies were performed in Asia, four in Europe, three in Africa, and one in America. Twelve studies had a case-control design, two were cohort studies, and two had a cross-sectional design. The main characteristics of each included study are summarized in online supplemental table 1.

Pooled effect of ghrelin levels on cases and controls

The pooled results (based on 20 studies) showed that there were no significant differences in ghrelin levels between cases and controls (SMD=−0.61, 95% CI −1.38 to 0.16; p=0.120; I^2=96.9%, p<0.001). Forest plots in figure 2A indicate the pooled SMD and each study on the ghrelin concentrations in cases and controls.

Sensitivity analyses for ghrelin levels showed that after the exclusion of the study by Kilic *et al*,[38] the pooled effect was changed (SMD=−0.78, 95%CI −1.55 to –0.03). In addition, the lower and higher pooled effects for our outcomes, after the one-by-one exclusion of the studies, are shown in figure 3.

Subgroup and meta-regression analyses

In subgroup analyses of ghrelin levels, we found that the levels of ghrelin were decreased in the diabetes/metabolic syndrome (MetS) stratum compared with those without diabetes/MetS comorbidities. The blood concentrations of ghrelin in the CAD stratum were significantly reduced, whereas these concentrations increased in other disease strata. The results of the continent subgroup analysis revealed that ghrelin levels decreased significantly in the Africa and Asia strata but increased in the Europe stratum (online supplemental table 2). Moreover, the findings of univariate meta-regression analyses based on total sample size, publication year, and the quality score showed that none of the moderator variables had a statistically significant effect on ghrelin levels (p≥0.05).

Combined markers

Regarding new combined markers, the findings revealed a significant decrease in the SMD of the ghrelin/TC ratio

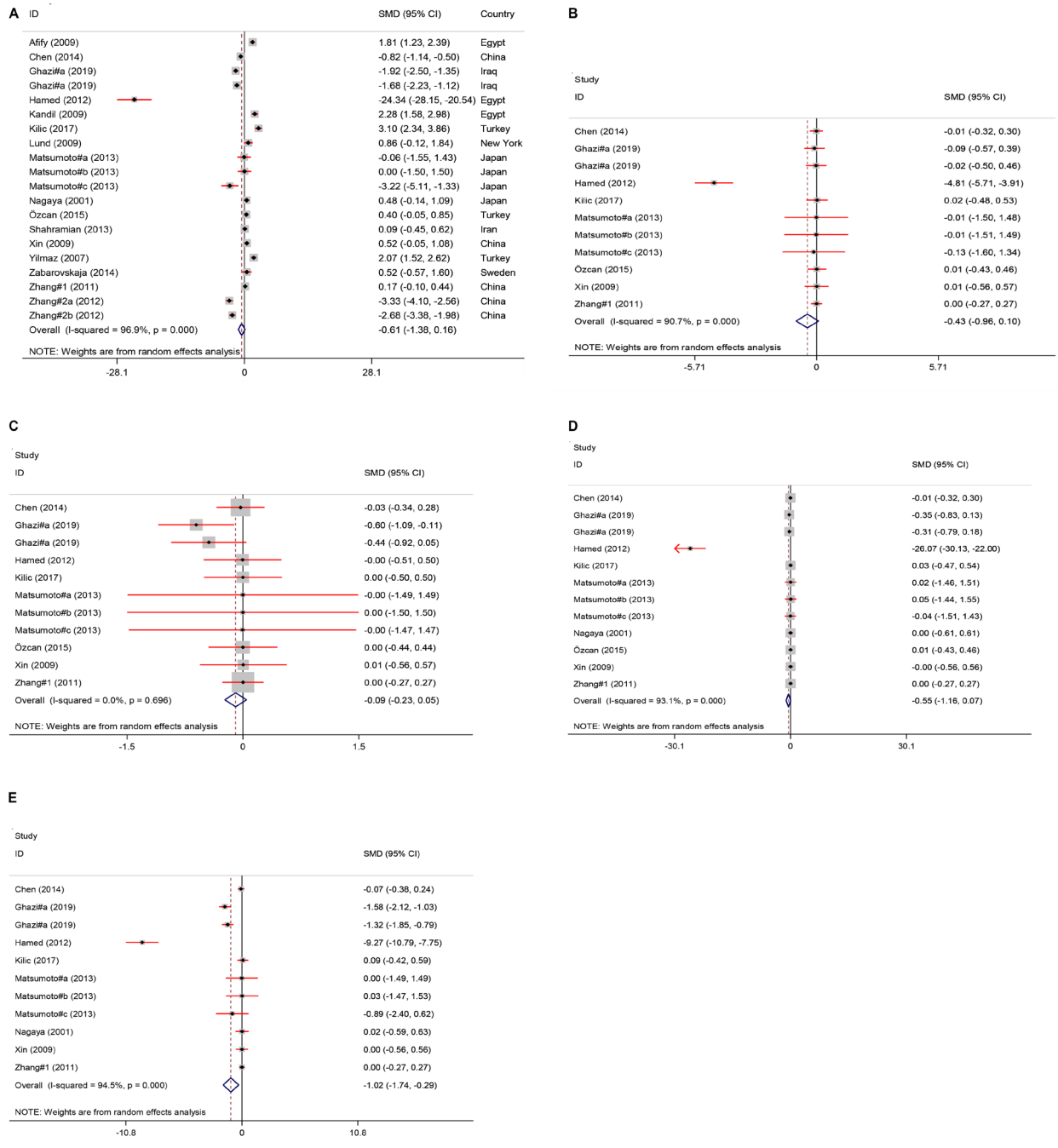

Figure 2 The forest plots of pooled estimates of SMDs of circulating ghrelin, ghrelin/HDL-c, ghrelin/LDL-c, ghrelin/TG, and ghrelin/TC levels between patients with CVDs and controls. CVD, cardiovascular disease; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; SMD, standardized mean difference; TC. triglyceride.

(−1.02; 95% CI −1.74 to −0.29, p=0.000; I^2=94.5%). However, no significant differences were found in the SMD of the ghrelin/HDL-c ratio (−0.43; 95% CI −0.96 to 0.10, p=0.000; I^2=90.7%), ghrelin/LDL-c ratio (−0.09; 95% CI −0.23 to 0.05, p=0.696; I^2=0.000%), and ghrelin/TG ratio (−0.55; 95% CI −1.16 to 0.07, p=0.000; I^2=93.1%) in cases with CVDs compared with the control group (figure 2B–E).

Subgroup and meta-regression analyses

In subgroup analyses of ghrelin/TC, ghrelin/HDL-c, and ghrelin/LDL-c levels, there were no significant changes in different strata, while the heterogeneity of some strata decreased. We found that the levels of ghrelin/TG were significantly decreased in the serum stratum compared with plasma. Moreover, ghrelin/TG blood concentrations in the CAD stratum were significantly reduced, whereas the differences in ghrelin/TG concentrations in other heart disease strata remained non-significant (online supplemental table 2).

To detect the possible source of between-study heterogeneity, a random-effects meta-regression was performed by the mean age and sample size of the intervention group subjects.

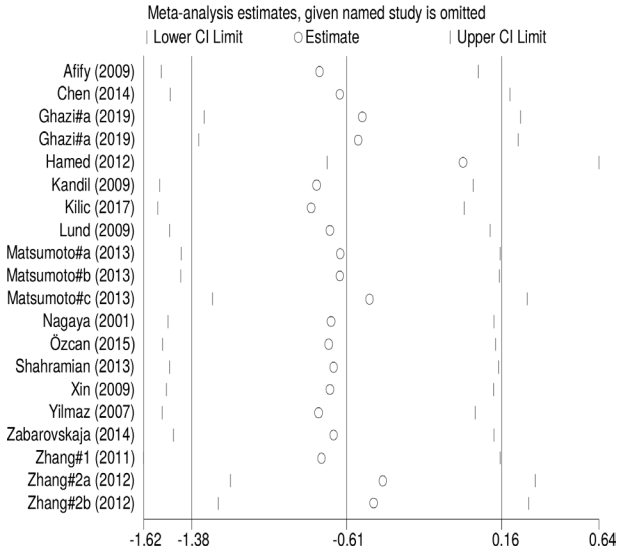

Figure 3 The sensitivity analysis results for SMDs of circulating ghrelin levels between patients with CVDs and controls. CVDs, cardiovascular diseases; SMD, standardized mean difference.

We evaluated age as a source of between-study heterogeneity for ghrelin/TC (coef: 0.15, 95% CI=(0.07 to 0.22), p=0.001), ghrelin/HDL-c (coef: 0.07, 95% CI=(0.02 to 0.11), p=0.005), and ghrelin/TG (coef: 0.39, 95% CI=(0.17 to 0.60), p=0.002), but not for ghrelin/LDL-c (coef: 0.00, 95% CI=(−0.00 to 0.01), p=0.35). However, we did not find a correlation between the sample size and ghrelin/TC (coef: 0.00, 95% CI=(−0.02 to 0.03), p=0.88), ghrelin/HDL-c (coef: 0.00, 95% CI=(−0.01 to 0.16), p=0.93), ghrelin/LDL-c (coef: 0.00, 95% CI=(−0.00 to 0.00), p=0.32), and ghrelin/TG (coef: 0.00, 95% CI=(−0.07 to 0.07), p=0.99).

Publication bias

Visual-filled funnel plots and Egger's test were used to evaluate the potential publication bias across the included studies (online supplemental figure a). However, Egger's tests indicated no significant evidence of publication bias for ghrelin levels (coef=−2.43, p=0.387).

DISCUSSION

The incidence of CVDs, as the main cause of death globally, is on the rise as the prevalence of risk factors for these diseases, such as diabetes, hypertension, smoking, dyslipidemia, and abdominal obesity is increasing in previously low-risk countries. Based on previous studies, the effects of these factors are higher in individuals with a genetic predisposition to CVDs.[1 2 49] Therefore, early diagnosis and treatment of CVDs is a critical issue.[3 6] Adipose tissue is known as an active endocrine and paracrine organ that secretes several bioactive mediators, including vaspin, chemerin, omentin-1, apelin, and ghrelin.[6 50] Thus, the dysregulation of these pro-inflammatory and anti-inflammatory adipokines in obesity might account for the link between obesity, insulin resistance (IR), and CVD.[51] The findings obtained from the present meta-analyses revealed that ghrelin levels were not significantly different between the two groups. Moreover, ghrelin levels were lower in patients with CVDs and diabetes/MetS. In addition, the blood concentrations of ghrelin in the CAD stratum were significantly reduced, whereas these concentrations increased in other disease strata. The results of the continent subgroup analysis revealed that ghrelin levels decreased significantly in the Africa and Asia strata but increased in the Europe stratum. In subgroup analyses of combined biomarkers, we found that the levels of ghrelin/TG were significantly decreased in the serum and CAD strata. Furthermore, meta-regression analyses revealed age as a source of between-study heterogeneity for ghrelin/TC, ghrelin/HDL-c, and ghrelin/TG.

As a gastrointestinal endocrine and cardioactive peptide, ghrelin is generated by cardiomyocytes and has diverse cardioprotective effects, including the inhibition of cardiomyocytes and endothelial cell apoptosis. It improves left ventricular function in ischemia/reperfusion.[11 52] According to the results of the present study, there was no statistically significant difference between patients with CVD and controls in terms of blood concentrations of ghrelin. Subgroup analyses based on diabetes/MetS comorbidities demonstrated that ghrelin levels were significantly lower in CVD patients with diabetes and MetS than controls. Ghrelin is known to modulate insulin secretion and is considered a promising molecular marker for IR. It was found that ghrelin contributes to the expression of the α-subunits and β-subunits of the insulin receptor. On the other hand, insulin inhibits basal and noradrenaline-triggered ghrelin secretion but does not affect ghrelin mRNA expression.[53] It has been reported that a lower ghrelin level is associated with higher severity of MetS indications including obesity, high blood pressure, and IR.[54] Although ghrelin levels were significantly lower in the CAD subgroup compared with the controls, there were significantly higher levels of ghrelin in patients with other CVDs than in controls. Since CADs are one of the main causes of death worldwide and lead to physical, mental, and social complications,[55] this adipocytokine may be considered a biomarker for distinguishing between patients with and without CAD. Ghrelin subgroup analyses also showed significantly lower ghrelin levels in Asian and African patients with CVD compared with controls. As shown in previous studies, the dynamics of ghrelin vary due to racial differences, which explains why ghrelin levels differed among subjects from various continents.[56] The findings on new combined markers indicated a significant decrease in the SMD of the ghrelin/TC ratio; however, no significant differences were observed in the SMD of the ghrelin/HDL-c ratio, ghrelin/LDL-c ratio, and ghrelin/TG ratio in cases with CVDs compared with controls. It has been demonstrated that both acylated ghrelin (AG) and unacylated ghrelin (UAG) bind to lipoproteins through different patterns of binding; AG binds to all lipoproteins and UAG binds more particularly to HDL-c. The binding of ghrelin to lipoproteins may modulate many of its cardiovascular functions. Any changes in the concentration of circulating lipoproteins may therefore impact the concentration of the free hormone available for receptor binding. Given the different patterns of lipoprotein binding, analysis of AG and UAG via separate immunoassays would be more informative.[57] Subgroup analyses of combined biomarkers revealed that the levels of ghrelin/TG were significantly decreased in the serum and CAD strata. More studies are required to evaluate the ghrelin/TG ratio in the serum of

patients with CVD. Moreover, the ghrelin/TG ratio could be considered a biomarker for patients with CAD, but due to high heterogeneity, further studies are needed to confirm this finding.

The strengths and limitations of this meta-analysis should be taken into consideration. This is the first comprehensive meta-analysis addressing adipocytokine ghrelin in relation to CVD. Subgroup analyses shed some light on the possible sources of heterogeneity in our study. Our meta-analysis has several limitations as well. Given that the studies included in this meta-analysis are heterogeneous, the results of this study should be interpreted with caution. In addition, the observed discrepancies in the present study could be attributed to gender variations, the use of laboratory kits with different characteristics, various atherosclerotic index evaluation methods, or the small sample size of the studies. Therefore, further large-scale studies are required to confirm the results.

CONCLUSION

The results of the current meta-analysis showed that there were no statistically significant differences between patients with CVD and the controls in terms of the blood concentrations of ghrelin. However, ghrelin levels were affected by the region and disease subtypes. Since ghrelin levels were significantly lower in the CAD subgroup compared with the controls, these levels may be considered a biomarker for distinguishing between patients with and without CAD. Based on these findings and as shown in a recent study, ghrelin can be proposed as a predictive biomarker for CVDs.[58] However, to bring ghrelin into clinical practice, it should exhibit further advantages in predicting cardiovascular risk. Moreover, additional studies are needed to prove that ghrelin indicates an independent relationship with cardiovascular pathology. It is worth mentioning that in addition to the genetic variants of the ghrelin gene, other factors, such as smoking, diet, percutaneous coronary intervention, or medication may affect ghrelin plasma levels. Hence, further research is required to find the risk factors affecting the levels of ghrelin in order to validate the current conclusions and to verify the biological significance of AG and UAG in health status.

Contributors All authors contributed to the study conception. TL, FM, and MZ did the literature search and screening. MA, AA, and SS data extraction. MN, SMS, and SS did the data synthesis, created the tables and figures, and wrote the manuscript. All authors contributed to the interpretation of the data and revision of the manuscript.

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