Original Article

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Biochemistry Section

Evaluation of Cardiometabolic Markers in *Helicobacter pylori* Infection: A Case-control Study

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ABSTRACT

Introduction: *Helicobacter pylori (H. pylori)* is a Gram negative bacterium that naturally colonises the gastric epithelium and causes chronic gastritis. *H. pylori* infection affects gastric physiology and alters the lipid metabolism pathways. High-sensitivity C-Reactive Protein (hs-CRP) is a useful marker for risk assessment of future cardiovascular events. Many studies have proposed a link between cardiometabolic markers like lipid profile and hs-CRP with *H. pylori* infection but very limited studies are available to explain the effect of *H. pylori* infection on these cardiometabolic markers.

Aim: To analyse the cardiometabolic markers (lipid profile and hs-CRP) in *H. pylori* infection.

Materials and Methods: This case-control study was conducted from November 2018 to June 2019 in the Department of Biochemistry in association with the Department of Surgery, Mysore Medical College and Research Institute, Mysore, Karnataka, India. Fifty cases and 50 control subjects were enrolled. Fasting Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C), hs-CRP, and Atherogenic Index of Plasma (AIP) were analysed. Statistical analysis was performed using Student's t-test.

Results: The gender distribution was almost same in the two groups. The mean age in the case group was 47.34±12.08 years, while that in the control group was 46.2±14.8 years. There was an increase in TC, LDL-C, VLDL-C, and TG in cases`, but it was not statistically significant. Serum HDL-C level was 34.59±9.79 mg/dL and 41.62±10.29 mg/dL for cases and controls respectively and it was statistically significant. hs-CRP level was significantly increased in the case group (5.51±4.59 mg/L) when compared to the control (2.63±2.0 mg/L). AIP was also significantly high in the cases (0.246±0.219) than the controls (0.106±0.22).

Conclusion: Significant decrease in HDL-C and increase in hs-CRP levels in cases show evidence of dyslipidaemia and atherogenic risk. hs-CRP also showed a significant correlation with AIP. Hence, these cardiometabolic markers may have a role in identifying individuals at higher risk for cardiovascular diseases in cases.

Keywords: Cardiovascular disease, Gastritis, Gram negative bacteria, Lipid profile

INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram negative bacterium that is widespread all over the world, infecting more than 50% of the world's population [1]. This causes chronic gastritis and peptic ulcer and is also a risk factor for developing gastric cancer, which is the second most frequent cause of cancer-related deaths [2]. *H. pylori* infection affects gastric physiology [3], and also alters pathways of lipid metabolism [1]. Several studies have identified that *H. pylori* infection might modify serum lipid concentrations, especially elevate Low Density Lipoprotein Cholesterol (LDL-C) and decrease High Density Lipoprotein Cholesterol (HDL-C) levels, which are the major risk factors for cardiovascular diseases [4-6]. HIgh-sensitivity C-Reactive Protein (hs-CRP) is a very valuable biomarker of inflammation and risk assessment for future cardiovascular events. hs-CRP assays measure very low levels of CRP in the blood. Many studies have proposed an association between serum hs-CRP levels and *H. pylori* infection [7-9]. But studies are lacking which categorise the *H. pylori* infected patients into low, moderate, and high-risk groups based on HDL-C and hs-CRP levels. In the present study, this categorisation was done to assess the risk for future cardiovascular events. Hence, the present study was undertaken to analyse the lipid profile and hs-CRP level in assessing the risk of cardiovascular diseases in *H. pylori* infection.

MATERIALS AND METHODS

The case-control study was conducted in the Department of Biochemistry, in association with the Department of Surgery, Mysore Medical College and Research Institute, Mysore, Karnataka, India, from November 2018 to June 2019. The ethical clearance was obtained from the Institutional Ethics Committee (IEC) (EC REG: ECR/134/Inst/KA/2013). Informed written consent was taken from all the patients.

Inclusion criteria: Patients with Functional Dyspepsia (FD) who attended the Surgery Outpatient Department (OPD) and needed evaluation for *H. pylori* infection were selected for the study. FD was defined as continuous or frequently recurring epigastric pain or discomfort centred in the upper abdomen for which no organic cause could be determined [10].

Exclusion criteria: Patients with chronic diseases like diabetes mellitus, chronic kidney disease, liver disease and known cases of cardiovascular and cerebrovascular diseases, patients on hypolipidaemic drugs, pregnant and lactating females and any diseases contraindicated to endoscopic procedure were excluded from the study.

Sampling size calculation: Based on the study done by Kansara GS et al., [7], the sample size was calculated considering the type I error to be 0.1 and type II error 0.2 (i.e. 80% power). The standard deviation in group 1 was considered 39.0 mg/dL and in group 2 was considered 42.9 mg/dL and expecting a mean difference of 20 mg/dL in the TG level between the two groups. The sample size was calculated to be 50 in each group (using the online sample size calculator). Finally, 50 *H. pylori*-positive and 50 *H. pylori*-negative subjects were included in the study.

An endoscopic gastric biopsy was obtained from all the study subjects and samples were sent for histopathological

examination. Based on the histopathological findings, the study subjects were divided into two groups: 50 biopsy-proven *H. pylori*-positive subjects with FD (case), and 50 *H. pylori*-negative with FD subjects (control). From all the subjects 3 mL of venous blood was collected in a fasting state and allowed to clot for 20 minutes at room temperature. The serum was separated by centrifugation at 1500 rpm for 10 minutes. Serum TC, TG, HDL-C and hs-CRP were estimated by methods as mentioned in the [Table/Fig-1] [11-13] using Cobas C311 fully automated chemistry analyser (Roche Diagnostics). Atherogenic Index of Plasma (AIP) was calculated using the above TG and HDL-C values by using the formula AIP=log (TG/HDL-C).

Parameters	Biochemical test method	Normal reference range/value
TC	CHOD-PAP method	Up to 200 mg/dL [11]
TG	GPO-PAP method	Up to 150 mg/dL [11]
HDL-C	Direct enzymatic method	40-60 mg/dL [11]
LDL-C	Calculation: Fredrickson Friedwald's formula {TC - HDL-C - (TG/5)}	Up to 100 mg/dL [11]
VLDL-C	Calculation: TG/5	Up to 40 mg/dL [11]
hs-CRP	Immunoturbidimetric method	<1 mg/L [12]
AIP	Calculation: AIP=log (TG/HDL-C) [11]	<0.11 [13]

[Table/Fig-1]: Normal reference range/value for lipid profile, hs-CRP and AIP. CHOD-PAP: Cholesterol oxidase phenol 4-aminoantipyrine peroxidase; GPO-PAP: glycerol phosphate oxidase peroxidase; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; VLDL-C: Very Low Density Lipoprotein Cholesterol (VLDL-C); hs-CRP: High-sensitivity C-Reactive Protein

The subjects were categorised into low, intermediate and high risk for future atherosclerosis based on HDL and hs-CRP levels. HDL-C levels <40 mg/dL was considered as low risk, 40-59 mg/dL as intermediate and >59 mg/dL as high risk group [11]. According to the American Heart Association, subjects were grouped into low, intermediate and high risk based on hs-CRP levels <1.0 mg/L; 1.0-3.0 mg/L and >3.0 mg/L respectively [7].

STATISTICAL ANALYSIS

Statistical analyses were performed with the GraphPad Instat version 3.1 software program. For comparison of variables with a normal distribution unpaired 2-tailed Student's t-test was used, whereas the Mann-Whitney U-test was used for variables with a skewed distribution. A p-value ≤0.05 was considered statistically significant.

RESULTS

Out of 50 cases, 26 (52%) were males and 24 (48%) were females and in the controls group 27 (54%) were males and 23 (46%) were females. Mean age of cases was 47.34±12.08 years and controls was 46.2±14.8 years and it was statistically not significant (p=0.67).

The mean serum TC, TG, LDL-C, and VLDL-C levels were higher among cases than those in the control group. But the difference was not statistically significant. There was a statistically significant decrease in the HDL-C levels among cases when compared to controls. Statistically significant high levels of AIP were observed among cases compared to controls. The mean value of the hs-CRP level among cases was significantly higher than in controls [Tables/Fig-2].

Out of 50, 39 (78%) of the cases had HDL-C <40 mg/dL and 11 (22%) had 40-59 mg/dL, whereas, only 16 (32%) of the controls had HDL-C <40 mg/dL and 32 (64%) subjects had 40-59 mg/dL but this difference was not statistically significant [Table/Fig-3].

Based on hs-CRP levels, subjects were divided into low, intermediate and high-risk group for the development of future atherosclerosis. Among cases 6 (12%) were at low-risk, 16 (32%) were at intermediate risk and 28 (56%) were at high-risk, whereas among the control group 12 (24%) were at low-risk, 22 (44%) were at intermediate risk and 16 (32%) were at high-risk. The difference was statistically significant only for high-risk group (p<0.001) [Table/Fig-4].

Parameters (normal range)	Cases (*H. pylori*-positive group) (n=50)	Controls (*H. pylori*-negative group) (n=50)	t-value	p-value
TC (upto 200 mg/dL)	161.10±37.96	157.32±37.22	0.503	0.615
TG (upto 150 mg/dL)	142.49±57.34	125.33±55.36	1.522	0.1321
HDL-C (40-60 mg/dL)	34.59±9.79	41.62±10.29	3.498	0.0007*
LDL-C (upto 100 mg/dL)	97.61±32.16	90.73±32.15	1.069	0.287
VLDL-C (upto 40 mg/dL)	28.31±10.6	25.61±11.95	1.195	0.234
AIP	0.246±0.219	0.106±0.22	3.191	0.002*
hs-CRP (<1 mg/L)	5.51±4.59	2.63±2.0	4.055	<0.0001*

[Table/Fig-2]: Comparison of lipid profile parameters among cases and controls. *statistically significant; Statistical test used- Student's t-test

HDL-C (mg/dL)	Cases		Controls		p-value
	No.	Mean±SD	No.	Mean±SD	
<40	39	30.6±6.2	16	29.5±5.7	0.536
40- 59	11	48.6±6.5	32	46.3±4.8	0.223
>59	0	-	2	62.8±3.9	-

[Table/Fig-3]: Risk stratification based on HDL-C values in cases and controls. Statistical test used- Student's t-test

Hs-CRP	Cases		Controls		p-value
	No.	Mean±SD	No.	Mean±SD	
Low risk (<1 mg/L)	6	0.61±0.20	12	0.69±0.16	0.399
Medium risk (1-3 mg/L)	16	1.8±0.57	22	1.9±0.53	0.587
High risk (>3 mg/L)	28	9.7±6	16	5±1.7	<0.001*

[Table/Fig-4]: Risk stratification of cases and controls based on hs-CRP. *statistically significant; Statistical test used- Student's t-test

There was a significant positive correlation between hs-CRP and AIP (r=0.245 and p=0.014). There was significant negative correlation between hs-CRP and HDL-C (r=-0.233 and p=0.02), LDL-C was also negatively correlated with hs-CRP but it was not significant (r=-0.043, p=0.672) [Table/Fig-5].

Lipid profile parameters	Pearson correlation with hs-CRP	p-value
HDL-C	-0.233	0.02*
LDL-C	-0.043	0.672
AIP	0.245	0.014*

[Table/Fig-5]: Correlation of hs-CRP with HDL-C, LDL-C and AIP. *Correlation is significant at the 0.05 level

DISCUSSION

The present study was undertaken to show the link between *H. pylori* infection and the risk of atherosclerosis. In the present study, among cases 52% were males and 48% were females and which did not show any significant gender distribution. This gender distribution was similar to a study done by Siddiqui B et al., where 373 (53%) were male and 325 (47%) were female patients [14].

In the present study, there was an increase in the mean values of TC, TG, and VLDL-C in cases when compared to controls

but these values were not statistically significant. HDL-C was decreased in the cases when compared to the controls, which was statistically significant. This was in agreement with a recent study done by Temesgen G et al., who found that the mean serum TG (125.28±61.67 mg/dL), TC (172.36±28.22 mg/dL) and LDL-C (109.73±32.78 mg/dL) levels were higher among cases than the mean serum TG (117.82±93.16 mg/dL), TC (164.56±30.04 mg/dL) and LDL-C (102.05±28.13 mg/dL) levels of controls. Serum HDL-C levels were 34.59±9.79 mg/dL and 41.62±10.29 mg/dL ($p<0.05$) for cases and controls, respectively [9].

No previous study has classified the subjects based on their HDL-C levels. Here, the study subjects were subgrouped into three groups based on the HDL-C levels. Among cases, 39 had HDL-C <40 mg/dL and 11 had 40-59 mg/dL whereas among controls only 16 subjects were at high-risk and 32 were at intermediate-risk and 2 were at low-risk. More subjects were at high-risk in cases but this was not statistically significant. According to Dobiasova M, AIP may be more closely associated with cardiovascular and cerebrovascular disease risk when compared to other lipoprotein cholesterol levels [15]. No previous studies were available to compare the AIP results. In the present study, AIP was high among cases (0.246±0.219) compared to controls (0.106±0.22) and it was statistically significant.

In the present study, hs-CRP levels were significantly increased in cases when compared to the controls, indicating that *H. pylori* seropositivity was associated with higher hs-CRP levels. These results were compatible with the studies by Ishida Y et al., [16], Altun E et al., [17], and Gen R et al., [18] and reported hs-CRP levels to be 8.2±4.2 ng/mL among *H. pylori*-infected subjects and 2.1±2.2 ng/mL among *H. pylori*-negative subjects ($p<0.05$). This was in agreement with one more study done by Temesgen G et al., where, the hs-CRP level for cases was 14.148±20.59 mg/dL and for *H. pylori*-negative was 3.51±8.39 mg/dL ($p<0.01$) [9]. In the present study, subjects were categorised into low, intermediate, and high-risk groups for the development of future atherosclerosis based on hs-CRP levels, out of 50 cases six were at low-risk, 16 were at intermediate-risk and 28 were at high-risk. This indicates that the majority of cases were at higher risk for developing cardiovascular diseases.

H. pylori infection causes chronic inflammation of the gastric mucosa with the systemic release of inflammatory cytokines, one of the contributory factors of atherosclerosis. Changes in lipid profile parameters may be a later consequence of this systemic inflammatory state. The pathophysiology underlying the alteration in the serum lipids was not fully clear. Different mechanisms were suggested, including the imbalance between synthesis and utilisation of plasma lipids, usage of lipids to restore damaged cell membranes, and interaction of cytokines and bacterial toxins with lipids [19]. Alterations in the composition and function of lipoproteins, due to decreased reverse cholesterol transport and increased oxidation of lipids occur by bacterial infection [20].

hs-CRP is mainly controlled by interleukin (IL)-6, which in turn is upregulated by other inflammatory cytokines like IL-1 and Tumor Necrosis Factor (TNF)-α [21]. There are several mechanisms by which hs-CRP can promote a proatherogenic environment in endothelial cells including the following, (i) decreasing prostacyclin and nitric oxide synthesis, (ii) increasing endothelin-1 concentration, and cell adhesion molecules such as monocyte chemoattractant protein-1 [16].

The present study correlated hs-CRP with most atherogenic lipid parameters used clinically like HDL-C and LDL-C and AIP and observed that HDL-C and AIP showed a statistically significant negative and positive correlation with hs-CRP respectively, which was concordant with another study [22], where a better correlation was observed for lipid indices like AIP than lipid profile. This shows the importance of lipid indices (which involve 2 atherogenic components of the lipid profile) as a better marker in assessing cardiovascular risk than the conventional lipid profile.

Limitation(s)

Other biochemical markers of atherogenicity like ferritin, and cytokines were not investigated, which constitutes the scope for further studies in this area.

CONCLUSION(S)

There was significant evidence of dyslipidaemia among cases compared to the controls as depicted by the decrease in HDL-C levels in cases. There was a statistically significant increase in hs-CRP levels in *H. pylori*-infected subjects. Hence, these cardiometabolic markers may have a role in identifying individuals at higher risk for cardiovascular diseases in cases.

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