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Haptoglobin: Can it be a Marker of Assessing Cardiovascular Risk in Type 2 Diabetes Mellitus?

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Abstract

- ***Background:***

Haptoglobin (Hp) is an acute phase protein that aids in clearance of free haemoglobin (Hb), thereby preventing Hb-mediated oxidative modification of serum or cellular proteins and lipids.

- ***Aims:***

We aim to correlate the levels of Haptoglobin with known lipid markers (HDL-C, LDL-C, Total-Cholesterol & Triglycerides) in type 2 diabetes.

- ***Methods:***

A case-control study was conducted enrolling 60 patients of type 2 diabetes (as per ADA-2011 criteria) and 60 age & sex matched healthy volunteers. Hp levels were assayed by ELISA based kit method. Lipid parameters were estimated using standard kits on automated analyzer. Data analysis was done using SPSS software.

- ***Results:***

Serum Hp levels were found to be significantly increased in type-2 diabetic patients (163.3±1.92 ng/ml) as compared to controls (108.7±2.68 ng/ml), p<0.001. Mean levels of serum HDL-C was found to be significantly lower in cases, p<0.001. A significant inverse correlation was observed between Hp levels and HDL-C concentration in type 2 diabetics (r = -0.53, R^2 = 0.28, p < 0.001). A significant positive correlation was observed between Haptoglobin and LDL-Cholesterol concentrations (r = 0.37, R^2 = 0.14, p = 0.004) and between levels of Haptoglobin and total cholesterol (r = 0.34, R^2 = 0.11, p = 0.008). But it was observed that correlation between Triglycerides and Haptoglobin was insignificant (r = 0.17, R^2 = 0.03, p = 0.205).

- ***Conclusion:***

Correlation of higher Haptoglobin levels with an atherogenic lipid profile suggests that serum Haptoglobin can be used as a test for CVD risk assessment in type 2 diabetics.

Keywords:- *Diabetes Mellitus, Acute phase protein, HDL, Lipids, Haptoglobin.*

I. INTRODUCTION

Diabetes mellitus has spread globally with around 382 million people affected by it in 2013 and expected to double by 2035 according to International Diabetes Federation [1]. The data suggest that this ever-rising illness affects 80% people in low and middle-income countries. More than 170 million people are still undiagnosed and a person dies from diabetes about every six seconds.[1] In India, it has been estimated that around 62 million people live with type 2 diabetes mellitus (DM) which is expected to rise to 101 million by 2030.[2] Studies have suggested that the rate of cardiovascular mortality and morbidity due to diabetes is almost double in India than in the U.S.[3] The role of oxidative stress has been recently documented in the pathogenesis of diabetes and related cardiovascular complications.[4] An early assessment of cardiovascular disease (CVD) risk is advocated in type 2 diabetics to initiate necessary circumspection.

Haptoglobin is a plasma protein that binds to free-hemoglobin released during low grade intravascular hemolysis. Thereby it inhibits free hemoglobin-induced oxidative damage and may have a role in pathological conditions associated with inflammation and oxidative stress like type 2 diabetes. Haptoglobin (Hp) is an acute phase protein synthesized in liver and fat tissue. It forms a complex with free hemoglobin in circulation which is rapidly cleared by liver and macrophages. Free hemoglobin (Hb) gives rise to toxic effects by generating reactive oxygen species like hydroxyl ions during fenton's reaction to contribute to oxidative stress seen in patients of type 2 diabetes. Moreover, it has been observed that the levels of free Hb increases in individual with DM due to stress mediated RBC lysis. Among the two proteins of Hp (Hp1-1 and Hp2-2), Hp1-1 has been documented to have higher antioxidant and endothelial protective function as compared to Hp2-2.[5] Due to increased Hb tethering to HDL via Hp 2-2 in DM, Hp phenotype is significantly associated with coronary ED in DM individuals.[6] It has been observed that enhanced modification of lipoproteins especially HDL-C (high density lipoprotein cholesterol) is a major factor

contributing to the accelerated atherosclerosis seen in DM patients.[7] Serum HDL-cholesterol levels have been correlated positively with the reduced incidence of atherosclerotic complications.[8] The primary mechanism by which HDL-C exerts a protective role is through reverse transport of cholesterol by promoting its efflux from macrophages. In DM, the athero-protective role of HDL-C is impaired along with its reduced plasma concentrations.[9,10] Several hypotheses have been postulated to explain the impaired role of HDL in DM. One of them is oxidative modification of ApoA-I which impairs the efflux of cholesterol from macrophages through LCAT and ATP binding cassette protein pathways.[11] Another theory says that Haptoglobin binds to ApoA1 to inhibit its PLTP (Phospholipid transfer protein) enzyme inducing activity which affects reverse cholesterol transport.[12] Higher circulating levels of Low density lipoprotein-Cholesterol (LDL-C) and total Cholesterol have classically been associated with increased CVD risk. Haptoglobin works as an antioxidant to prevent the ominous modification of lipids (especially HDL-C & LDL-C) and proteins mediated through free radicals in diabetes mellitus.

In a study on Chinese patients with T2D, Hp 1-1 phenotype was found associated with increased risk of incident AMI, independent of traditional risk factors. Hp phenotyping may allow for identification of T2D individuals at higher risk for onset of AMI.[13] The increased oxidative stress seen in both in vitro and in vivo patients with DM and the Hp 2-2 genotype suggests antioxidants as a potential target for mitigating CVD risk among this group. Vitamin E administration is a potential treatment that could provide an affordable, accessible and effective benefit to patients with high oxidative stresses on their CV system.[14]

The aim of the present study was to estimate serum levels of Haptoglobin in type 2 diabetic subjects & healthy controls and correlate their levels with circulating blood lipids (HDL-C, LDL-C, Cholesterol-total & Triglyceride) in the case group to evaluate a possible role of Haptoglobin for CVD risk assessment in type 2 DM as dyslipidemia itself is a risk factor for cardiovascular disease.

II. MATERIALS AND METHODS

An analytical, case control study was conducted in the Department of Biochemistry of a tertiary care hospital with the enrolment of 120 subjects after an informed written consent. The methods used were in agreement with the institutional ethical committee and the Declaration of Helsinki.

Inclusion criteria: Case group was formed by sixty (age group, 35-60 yrs) newly diagnosed patients of diabetes mellitus (based on ADA 2011 criteria) selected from the hospital medical OPD.

Control group consisted of apparently healthy volunteers of comparable age group and gender (n=60). They visited the health care centre for comprehensive health check-up or were volunteers for blood donations. None of them had any history of intake of dietary supplements or multivitamins. All subjects underwent the same protocol.

Exclusion criteria: Subjects with history of cerebrovascular disease, acute or chronic debilitating illness, pregnant or nursing women, dialysis and peripheral vascular disease were excluded from the study.

Sample collection: Fasting venous sample was collected under sterile conditions in NaF-EDTA evacuated vials (BD Vacutainer®) for plasma and in plain vacutainer (no additives) for serum. Biochemical analysis was performed in the Clinical Biochemistry Laboratory using standard kits from Randox laboratories (UK) on automated clinical chemistry analyzer (CX series, Beckman Coulter, Inc. US). All analysis was run in duplicates and average values were used.

Biochemical analysis: Plasma glucose levels were estimated by Glucose oxidase method (Randox: Glucose GOD-PAP, GL3981). Serum HDL-Cholesterol estimation was done by direct clearance method which works by completely removing all non-HDL components including abnormal lipoproteins (Randox: HDL-C, CH3811). Serum total Cholesterol was estimated by cholesterol oxidase method (Randox: Total Cholesterol CHOD-PAP, CH3810). Serum Triglycerides were estimated using the Glycerol-phosphate oxidase based colorimetric method (Randox: Triglycerides (GPO-PAP), CH 3823). Serum LDL-cholesterol was calculated using the Friedwald's formula [LDL-C = Total Cholesterol- (HDL-C + VLDL-C)] where VLDL-C = TG/5).

Haptoglobin assay: A standard solid phase sandwich ELISA kit was used for estimation of Haptoglobin from R & D systems (Human Haptoglobin Quantikine™ ELISA Kit: R&D Systems™, DHAPG0). As per manufacturer instructions, the protocol was followed and all samples, standard and controls (10 µl) were run in duplicates taking all precautions mentioned. A chromogenic substance was added to read absorbance at 405 nm after a 4.5 hr procedure. Standard curve was plotted by using standards of known concentrations to identify the values of samples. Sensitivity of the assay was 0.529 ng/ml. Assay range 3.13-200 ng/ml. <0.5% cross reactivity observed. Inter-assay precision of less than 8% and intra-assay less than 6%.

Statistical analysis: Statistical package for Social Science statistical software version 20 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Group data has been presented as mean values ± Standard Error of Mean (S.E.M). Quantitative data was assessed using independent sample t-test. To assess correlation between study variables Pearson's correlation analysis was used. A value of $p \leq 0.05$ was considered statistically significant.

III. RESULTS

The baseline characteristics of the two groups were comparable with respect to age and sex as shown in Table 1. Biochemical analysis revealed that serum Haptoglobin levels were significantly higher in cases (153.3 ± 1.92 ng/ml) as compared to controls (98.7 ± 2.68 ng/ml), p<0.001 as shown in Figure 1. Mean levels of serum HDL-C in type 2 diabetic group was (37.3 ± 0.77 mg/dl) compared to controls (44.6 ± 0.85 mg/dl), p<0.001 as shown in Figure 2. Serum LDL-C levels were found to be higher in cases (78.4 ± 4.89 mg/dl) than controls (75.9 ± 3.72 mg/dl) but the finding was not statistically significant (p =0.681). Similarly mean concentration of Total Cholesterol in the cases was insignificantly higher than controls (148.9 ± 5.02 mg/dl vs. 146.2 ± 3.54, p =0.659). However the difference in serum triglycerides was significant (167.2 ± 9.79 vs. 125.3 ± 4.90, p< 0.001).

Pearson's correlation analysis performed separately between Haptoglobin and each of the blood lipid markers as shown in Figure 3. HDL-Cholesterol, which is considered as an important independent risk factor for CVD assessment, depicted a significant and negative correlation with serum haptoglobin levels ($r = -0.53$, $R^2 = 0.28$, $p < 0.001$). A significantly positive correlation was observed between Haptoglobin and LDL-Cholesterol concentrations ($r = 0.37$, $R^2 = 0.14$, $p = 0.004$). Similarly, a positive and significant correlation was found between levels of haptoglobin and total cholesterol($r = 0.34$, $R^2 = 0.11$, $p = 0.008$). But it was observed that correlation between Triglycerides and haptoglobin was insignificant ($r = 0.17$, $R^2 = 0.03$, $p = 0.205$).

IV. DISCUSSION

In an attempt to facilitate early CVD risk assessment in type 2 diabetics, we investigated the possible role of Haptoglobin, a known anti-inflammatory and antioxidant plasma protein. As diabetes mellitus has multi-factorial etiology, the precise role of inflammation in the pathogenesis of DM is still under evaluation. The rise in oxidative stress due to uncontrolled or prolonged hyperglycemia results in oxidative modification and impaired functioning of various proteins and lipids in patients of type 2 DM which contributes to various vascular complications seen in DM.

In our study, we found significantly high levels of Haptoglobin in patients of type 2 DM as compared to their respective controls. Our results were in agreement with the findings of work done in other studies.[15,16,17] As Haptoglobin is a positive acute phase protein and aids in removal of free Hb mediated oxidative damage, the rise in its levels is suggestive of enhanced oxidative stress. Moreover, Haptoglobin is a known modulator of erythrocytic aggregation and it may contribute to vascular complications through this mechanism as well.[18] It has been observed that in diabetes, the removal of Hp-Hb complex from the circulation is impaired.[19] Several studies have investigated the link between Hp polymorphisms and CVD risk in DM.[20,21] A recent long term study stressed on the significance of vitamin E as an antioxidant in preventing the progression and development of complications associated with DM, thereby further confirming the role of oxidative stress in DM.[19] Studies have shown that the two alleles of Hp: Hp 1-1 and Hp 2-2 differ in their binding capacity with non-heme iron and may mediate different degrees of protection in oxidative stress.[22] Metabolic syndrome, hypertension and hyperglycemia have also been linked with raised Haptoglobin levels.[23] Another study showed that Hp 2-2 is an independent risk factor for myocardial infarction.[24]

We found significantly reduced levels of serum HDL-C in cases as compared to controls as observed in some other studies as well.[22,25] HDL-C is a known predictor for cardiovascular disease in DM patients. It has been proposed that oxidative modification of serum lipoproteins (HDL-C and LDL-C) is an important predictor for atherosclerosis in type 2 DM.[26] Enhanced non-heme iron mediated oxidative stress impairs the functioning of lipoproteins through several mechanisms. Studies have detected substantial amounts of Hb associated with HDL-C in DM patients and it clearance is also impaired.[7,27] Pro-oxidant Hb associated with HDL-C in DM results in oxidative modification of HDL-C associated lipids and proteins like ApoA1 and oxidative modification of HDL-C associated enzymes like paraoxonase.[28] Studies have shown that there is reduced clearance of this Hp-Hb complex bound to HDL-C.[19] In DM, not only the amount of HDL-C is reduced but its effect in controlling atherosclerosis through reverse transport of cholesterol is also impaired.

In this study, a significant correlation was observed between Hp & HDL-C levels and between Hp & LDL-C levels in cases. Speculated mechanism for the dysfunction of HDL-C by Haptoglobin is through inhibition of LCAT (Lecithin Acyl Transferase) mediated reverse cholesterol transport[29] and LCAT plays a role in maturation of HDL-C.[30] As observed in our study, the higher levels of Haptoglobin correlated with reduced levels of HDL-C and increased levels of LDL. Another study revealed that in DM, Phospholipid transfer protein (PLTP) activity is affected which correlates well with HDL-C levels.[12] PLTP activity is also dependent on Apo-A1 which is affected by Haptoglobin.[31,32] Oxidative modification of LDL results in its enhanced deposition in the endothelium and atherosclerotic lesions. Our study showed a negative correlation btw Hp and TG levels similar to the work of Adams et al.[23], although it was not statistically significant. The mechanism for this association is still unclear. We also observed a significant positive correlation of Hp with cholesterol suggestive of its enhanced modification and reduced clearance from circulation.

Limitations of study: Small sample size, case control study are limitations of this study.

V. CONCLUSION

Our findings suggest that newly diagnosed type 2 diabetics have a lipid profile which is significantly more atherogenic as compared to healthy controls. This could be either the cause or effect of a state of chronic low-grade inflammation and oxidative stress in type 2 DM. Serum Haptoglobin concentration is significantly higher in type 2 diabetics and positively correlates with an atherogenic lipid profile reflecting a warning sign and prompting circumspection. Higher levels of serum Haptoglobin in type 2 diabetics may be attributed to oxidative stress and low grade inflammation. Correlation of higher Haptoglobin levels with an atherogenic lipid profile suggests that serum Haptoglobin can be used as a test for CVD risk assessment in newly diagnosed type 2 diabetics.

REFERENCES

[1]. International Diabetes Federation. IDF Diabetes Atlas, 6th edn. Brussels, Belgium: International Diabetes Federation, 2013.
[2]. Whiting DR, Guariguata L, Weil C, Shaw J. IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Research and Clinical Practice. 2011;94(3):311-321.
[3]. World Health Organization, Non-Communicable Diseases in the South-East Asia Region: Situation and Response 2011, World Health Organization, New Delhi, India. 2011.
[4]. Suzuki D, Miyata T, Saotome N, Horie K, Inagi R, Yasuda Y, et al. Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions. J Am Soc Nephrol. 1999;10:822-832.
[5]. Levy AP, Hochberg I, Jablonski K, Resnick HE, Lee ET, Best L, et al. Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the Strong heart Study. J Am Coll Cardiol. 2002;40:1984-1990.
[6]. Rabea A, Levy AP,Levy NS, Asleh A, Goldenstein H, Segol I, et al. Haptoglobin Phenotype Is Associated With High-Density Lipoprotein–Bound Hemoglobin Content and Coronary Endothelial Dysfunction in Patients With Mild Nonobstructive Coronary Artery Disease . Arteriosclerosis, Thrombosis, and Vascular Biology. 2019;39:774–786
[7]. Asleh K, Marsh S, Shilkrut M, Binah O, Guetta J, Lejbkowicz F, et al. Genetically determined heterogeneity in haemoglobin scavenging and susceptibility to diabetic cardiovascular disease. Circ Res. 2003; 92(11):I193-200.
[8]. Boden WE. High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the Veterans Affairs High-Density Lipoprotein Intervention Trial. Am J Cardil. 2000;86:19-22.
[9]. Kontush A, Chapman MJ. Antiatherogenic small, dense HDL—guardian angel of the arterial wall? Nat Clin pract Cardiovasc Med. 2006;3:144-153.
[10]. Nobecourt E, Jacqueminet S, Hansel B, Chantepie S, Grimaldi A, Chapman MJ, et al. Defective antioxidative activity of small, dense HDL particles in type 2 diabetes: relationship to elevated oxidative stress and hyperglycemia. Diabetologia. 2005;48:529-538.
[11]. Salvatore A, Cigliano L, Bucci EM, Corpillo D, Velasco S, Carlucci A, et al. Haptoglobin binding to apolipoprotein A-1 prevents damage from hydroxyl radicals on its stimulatory activity of the enzyme lecithin-cholesterol acyl-transferase. Biochemistry.2007;46:11158-11168.
[12]. Henderson RJ, Wason KM, Leon CG. Haptoglobin inhibits phospholipid transfer protein activity in hyperlipidemic human plasma. Lipids in Health and disease. 2009,8:27.
[13]. Gurung, R.L., Yiamunaa, M., Liu, S. et al. Association of haptoglobin phenotype with incident acute myocardial infarction in Chinese patients with type 2 diabetes. Cardiovasc Diabetol . 2019; 18: 65
[14]. Asleh R, Briasoulis A, Berinstein EM, Wiener JB, Palla M, Kushwaha SS et al. Meta-analysis of the association of the haptoglobin genotype with cardiovascular outcomes and the pharmacogenomic interactions with vitamin E supplementation. Pharmgenomics Pers Med. 2018; 11: 71–82.
[15]. Asleh R, Levy AP. In vivo and in vitro studies establishing haptoglobin as a major susceptibility gene for diabetic vascular disease. Vasc health risk Manaq. 2005;1(1):19-28.
[16]. Levy AP. Haptoglobin: a major susceptibility gene for diabetic cardiovascular disease. Isr Med Assoc J. 2004; 6(5):308-310.
[17]. Mohieldein A, Alzohairy M, Hasan M, Khan AA. Inflammatory markers and haptoglobin polymorphism in Saudi with non-insulin –dependent diabetes mellitus. Global Journal of Health science. 2013;5(1).
[18]. Rema M, Mohan V, Snehlata C. Acute phase proteins in diabetic nephropathy. Indian J Ophthal. 1996:44(2);83-85.
[19]. Asleh R, Blum S, Litman SK, Alshiek J, Lotan RM, Asaf R et al. Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2-2 genotype. Diabetes. 2008;57:2794-2800.
[20]. Adams NJ, Cox JA, Freedman IB, Langefeld DC, Carr JJ, Bowden WD. Genetic analysis of haptoglobin polymorphisms with cardiovascular disease and type 2 diabetes in the diabetes heart study. Cardiovasc Diabetol. 2013;12:31.
[21]. Costacou T, Ferrell RE, Orchard JT. Haptoglobin: a determinant of cardiovascular complication risk in type-1 diabetes. Diabetes. 2008;57:1702-1706.
[22]. Asleh R, Lotan MR, Aviram M, Hayek T, Yulish M, Levy EJ et al. Haptoglobin genotype is a regulator of reverse cholesterol transport in diabetes in vitro and in vivo. Circ Res. 2006;99:1419-1425.
[23]. Hamalainen P, Saltevo J, Kautiainen H, Mantyselka P, Vanhala M. Erythropoietin, ferritin, haptoglobin, haemoglobin and transferring receptor in metabolic syndrome: a case control study. Cardiovasc Diabetol. 2012;11:116.

[24]. Gogishvili AV, Kavtaradze VG, Mamaladze GT, Arutiunova MS and Takadze GSh. Haptoglobin phenotype distribution in patients at high risk of developing myocardial infarct. Kardiologiia. 1985;25:55-58.
[25]. Mustafa S, Vukovich T, Prikoszovich T, Winzer C, Schneider B, Esterbauer H. Haptoglobin phenotype and gestational diabetes. Diabetes Care. 2004;27:2103-2107.
[26]. Hansel B, Giral P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ, et al. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. J Clin Endocrinol Metab. 2004; 89:4963-4971.
[27]. Koch W, Latz W, Eichinger M, Roguin A, Levy AP, Schomig A, et al. Genotyping of common haptoglobin polymorphism Hp ½ based on the polymerase chain reaction. Clin Chem. 2002;48:1377-1382.
[28]. Navab M, Ananthaaramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fogelman AM. Mechanisms of disease: proatherogenic HDL-an evolving field. Nat Clin Prac. 2006;2:504-511.
[29]. Balestrieri M, Cigliano L, Simone ML, Dale B, Abrescia P. Haptoglobin inhibits lecithin-cholesterol acyltransferase in human ovarian follicular fluid. Mol Reprod Dev. 2001;59(2):186-91.
[30]. Rader DJ. Molecular regulation of HDL metabolism and function: implications for novel therapies. J Clin Invest. 2006;116:3090-3100.
[31]. Colhoun HM, Taskinen MR, Otvos JD, Van Den BP, O'Connor J, Van TA. Relationship of phospholipid transfer protein activity to HDL and apolipoprotein B-containing lipoproteins in subjects with and without type I diabetes. Diabetes. 2002;51(11):3300-3305.
[32]. Cheung MC, Albers JJ. Active plasma phospholipid transfer protein is associated with apoA-I-but not apoE-containing lipoproteins. *J Lipid Res*. 2006;47(6):1315-1321.

Parameter	Type 2 Diabetes Mellitus cases (n=60)	Healthy controls (n=60)	P value
Age [yrs] (mean± SD)	57.88 ±8.67	59.12 ±8.42	0.470
Male (%)	26 (42%)	28(46%)	0.687
Female (%)	34 (58%)	32(54%)	

Table 1:- Demographic Profile of study population

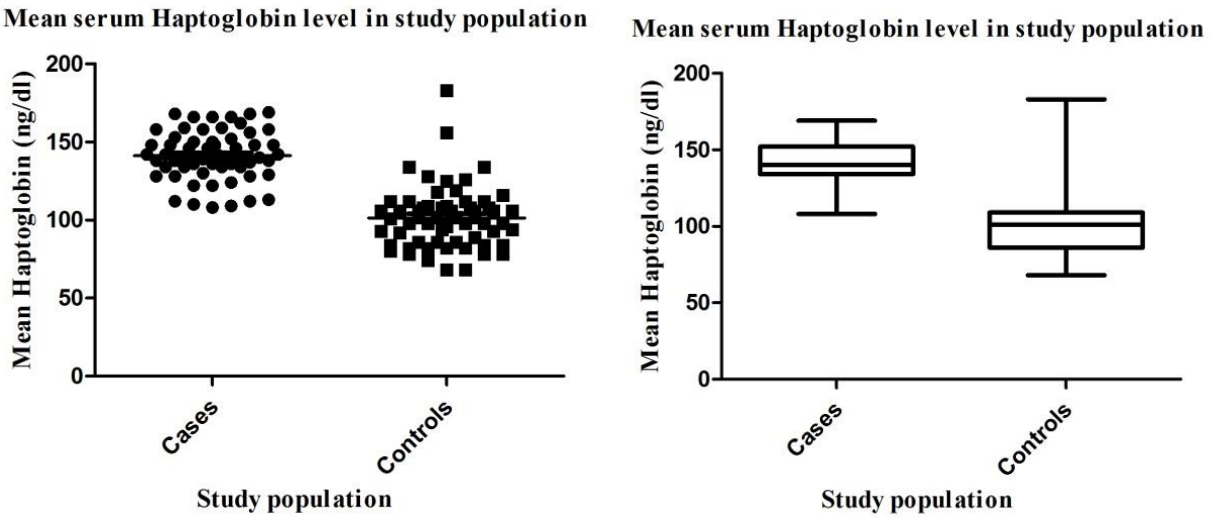

Fig 1:- Mean serum haptoglobin level in study population.

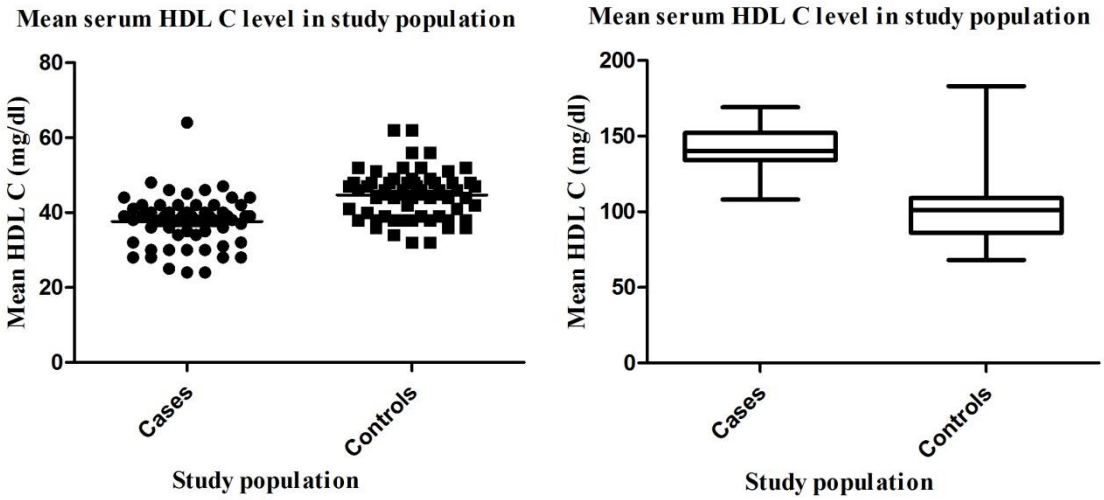

Fig 2:- Mean serum HDL-Cholesterol level in study population.

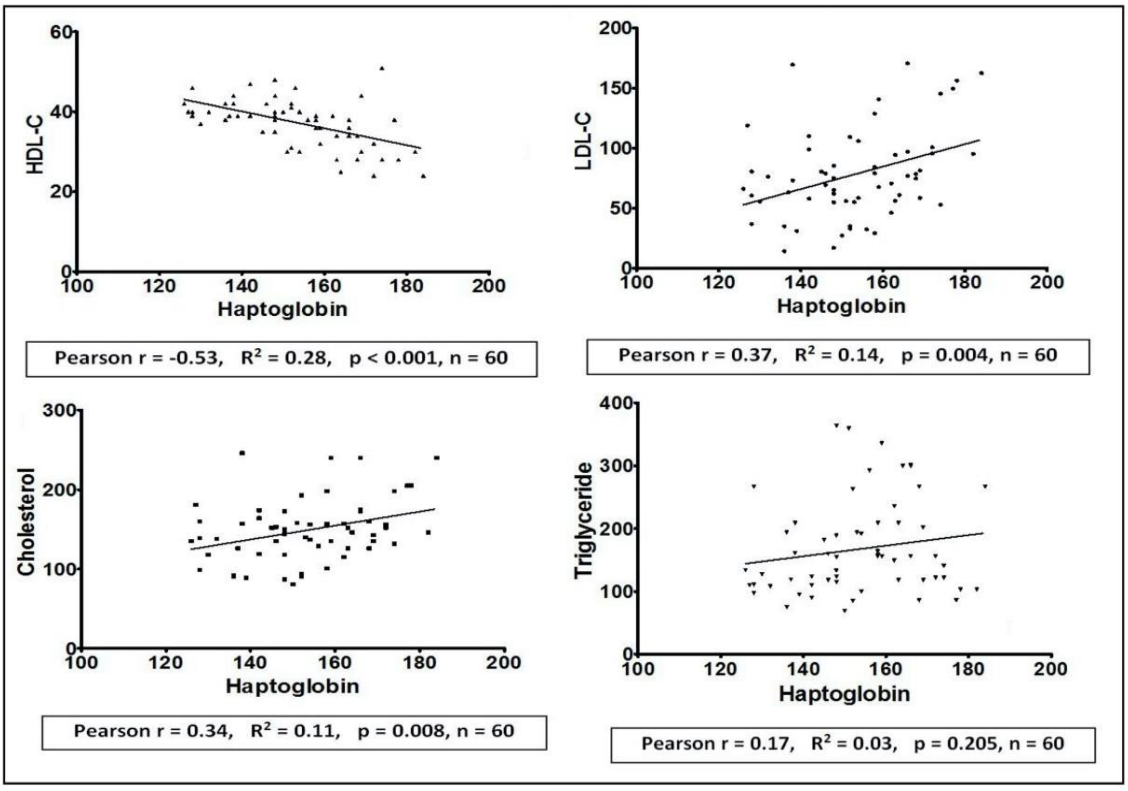

Fig 3:- Correlational analysis among study variables.