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Research Article

SERUM HS-CRP AND MAGNESIUM IN TYPE 2 DIABETIC PATIENTS WITHOUT AND WITH COMPLICATIONS

***Mirza Sharif Ahmed Baig[1], Manjushree Sugoor[1] and Khwaja Nawazuddin Sarwari[2]**
[1]Department of Biochemistry, KBN Institute of Medical Sciences, Gulbarga, India
[2]Department of Physiology, KBN Institute of Medical Sciences, Gulbarga, India
**Author for Correspondence*

ABSTRACT
Type 2 diabetes mellitus has become a leading cause of morbidity and mortality world over. Hs-CRP is a marker of low-grade inflammation and its level is raised in patients with type 2 DM. Magnesium homeostasis has been hypothesized to be a link between insulin resistance, type 2 diabetes mellitus, hypertension and CAD. The current study was undertaken in 90 subjects. 30 diabetics without complications (group I), 30 diabetics with complications (group II) and 30 non diabetics as normal control group (Group III). The aim of the study was to assess the serum levels of Hs-CRP and magnesium in type 2 DM patients without and with complications. Hs-CRP is significantly much higher in diabetic cases with complications compared to cases without complications. The elevation is significant in both the study groups when compared to controls. We found significantly far lower levels of serum magnesium in diabetic cases with complications when compared to cases without complications. The lowering is significant in both the study groups when compared to controls. TC, TG, LDL-C and VLDL-C levels where significantly elevated and HDL-C levels were significantly lower in cases of both the groups when compared to controls. There is an inverse correlation between the hs-CRP and magnesium levels. Hence it is concluded that the serum levels of Hs-CRP and magnesium appear to be useful as markers of diabetic complications and provide valuable information for proper medical intervention.

Keywords: *High Sensitive-C Reactive Protein, Type 2 Diabetes Mellitus, Serum Magnesium & Lipid Profile.*

INTRODUCTION
Although diabetes has been recognized, since antiquity and treatment of various efficacy have known in various regions since the Middle Ages, and in legend for much longer pathogenesis of diabetes has only been understood experimentally since about 1900. Diabetes mellitus, the most common endocrine disease is characterized by metabolic abnormalities and by long-term complications involving the eyes, kidneys, nerves and blood vessels. With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality in the foreseeable future.

The increase in the incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a western-style diet.

Pickup *et al.,* (2004) suggested an increasing interest in the involvement of low grade inflammation in the pathogenesis of type 2 diabetes. CRP is an inflammatory marker produced and released by the liver under the stimulation of cytokines such as tumor necrosis factor - α and interleukins 1 and 6. It has emerged as a powerful risk marker for cardiovascular disease. Inflammation has also been postulated to play a role in the pathogenesis of type 2 diabetes.

Nakanishi *et al.,* (2003) have suggested that an elevated level of CRP is associated with an increased risk of developing type 2 diabetes. Ford *et al.,* (1999) studied CRP levels are higher in people with diabetes compared with those without diabetes. Dehghan *et al.,* (2007) shown that CRP is independently associated with the development of diabetes.

Festa *et al.,* (2000) demonstrated that elevated levels of CRP are associated with obesity, insulin resistance and glucose intolerance, suggesting that inflammation is also involved in the etiology of type 2 diabetes.

Magnesium, an essential element in the mechanism of glucose transport across cell membrane and a component of various enzymes in carbohydrate oxidation. Tosiello *et al.,* (1996) studied that hypomagnesemia is a common feature in patients with type 2 diabetes.

Although diabetes can induce hypomagnesemia, magnesium deficiency has also been proposed as a risk factor for type 2 diabetes. The association between diabetes mellitus and hypomagnesemia is compelling for its wide-ranging impact on diabetic control and complications. Mc Nair *et al.,* (1978) shown that magnesium deficiency has been found to be associated with diabetic microvascular disease. Several mechanisms, including insulin secretion, binding and action have been proposed to explain the effect of intracellular or plasma magnesium on diabetes pathogenesis.

Magnesium homeostasis has been hypothesized to be a link between insulin resistance, type 2 diabetes, hypertension and cardiovascular diseases. It is also known that there is an inverse relationship between magnesium intake and the risk of diabetes.

The objective of this study was to assess the serum levels of hs-CRP and magnesium in type 2 diabetic patients without and with complications.

MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry KBN Institute of Medical Sciences Gulbarga. Clearance was obtained from the institutional ethical committee.

The study was carried out on 30 age and sex matched healthy controls and 60 type 2 diabetic patients who attended the outpatient and inpatient department of KBN Institute of Medical Sciences Gulbarga. A total 60 patients of type 2 diabetes mellitus between 40 – 70 years, which were divided into following groups.

Control group: Included 30 healthy, age and sex matched individuals.

Group I: Included 30 patients of type 2 diabetes without complications.

Group II: Included 30 patients of type 2 diabetes with proven complications, like CAD, retinopathy and neuropathy.

The diagnosis of type 2 diabetes mellitus was established with the recommended criteria's of American diabetes Association.

Inclusion Criteria

Patients in the age group of 40 – 70 years with type 2 diabetes without and with proven complications, like CAD, neuropathy and retinopathy were selected.

Exclusion Criteria

Patients with recent infectious disease, immunological disorders, taking diuretics and magnesium containing antacids, Surgeries, Burns, malabsorption syndrome, chronic diarrhoea, renal failure, pancreatitis, alcoholism, liver diseases, tuberculosis, thyrotoxosis, Osteoarthritis, Rheumatoid arthritis and all other inflammatory disorders were excluded from the study.

Informed consent was taken from patient and control subjects. A pre-structured and pre-tested proforma was used to collect the data. Baseline data including age and sex, detailed medical history including conventional risk factors, clinical examinations and relevant investigations including ECG, echocardiogram, nerve conduction test, fundoscopy etc were included as part of the methodology.

Laboratory Methods

Fasting venous blood samples were collected from cases and controls and the samples were centrifuged, serum was separated and stored at 4^0C. Mendall *et al.,* (1996) Serum high sensitive C-reactive protein was measured using the immunoturbidimetric CRP assay by using auto-analyzer (A_{25}Biosystem), based on the principle of agglutination reaction. Presence of CRP in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at wavelength between 505-578 nm. The increase in turbidity corresponds to the concentration of CRP in the test specimen.

Gindler *et al.,* (1971) Serum magnesium was estimated by Calmagite dye method by using auto-analyzer (A_{25}Biosystem). Magnesium reacts with the blue dye, calmagite, in alkaline medium to form red colored

complex which is measured at 530-550nm. The intensity of the color formed is directly proportional to the amount of magnesium in the sample. Protein interference and dye precipitation are avoided including the 9-ethylene oxide adduct of p-nonylphenol (Bion NE9) and Polyvinyl pyrrolidone (Bion pup). Calcium interference is avoided by preferential combination with EDTA and heavy metal interference is prevented by Potassium cyanide.

Estimation of Serum total cholesterol by COD-POD method, Serum triglycerides by Tinder's GPO-POD method and serum HDL cholesterol by Phosphotungstate method. Serum LDL cholesterol and VLDL cholesterol values were calculated by applying Friedewald's formula.

Serum Creatinine estimation was carried out using Jaffe's alkaline picrate method and blood urea was measured using Specific Urease method. FBS and PPBS were measured by GOD/POD method. Urine sample was analyzed for protein and sugar by using dipsticks.

Statistical Methods

Student t test/Chi-square test has been used to find the significance of homogeneity of study characteristics between three groups of patients. Analysis of variance has been used to find the significance of study parameters between three groups.

Results were expressed as mean $\pm$ SD, p values are obtained by using the post-hoc Turkey test.

Significant Figures

+ Suggestive significance $0.05<p<0.10$

* Moderately significant $0.01<p \leq 0.05$

** Strongly significant $p \leq 0.01$

Statistical software

The Statistical software namely SPSS 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

RESULTS AND DISCUSSION

A Comparative three-arm study with 30 in Controls, 30 in diabetic patients without complications and another 30 patients in Diabetics with complications is undertaken to study the Biochemical parameters

Table 1: Hs-CRP and Sr.Mg in the three study groups

Study parameters	Controls	DM without complications	DM with Complications
hs-CRP mg/L	0.72±0.45	2.27±1.14	3.55±0.86
Sr.Mg mg/dl	2.17±0.35	1.61±0.41	1.29±0.31

Results are presented in Mean±SD

Study parameters	Controls Vs DM without complications	Controls Vs DM with complications	DM without Complications Vs DM with Complications	Effect size	
				Controls Vs DM without complications	Controls Vs DM with complications
hs-CRP mg/L	<0.001**	<0.001**	<0.001**	1.77	3.52
Sr.Mg mg/dl	<0.001**	<0.001**	0.003**	1.45	2.63

p values are obtained by using the Post-hoc Tukey test.

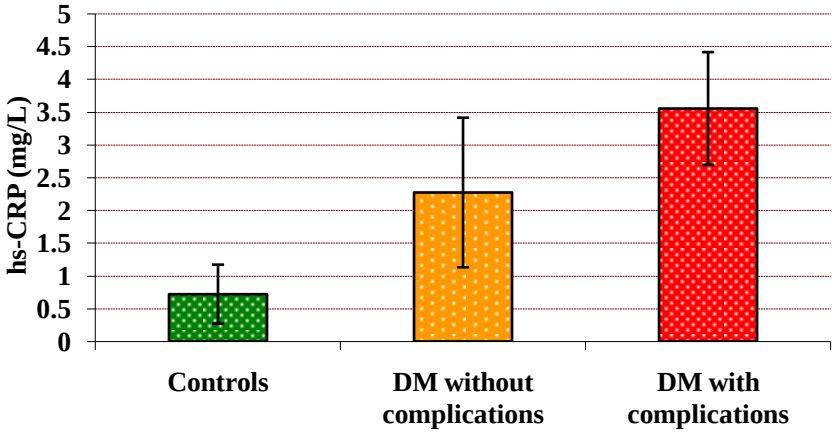

Figure 1a: Hs-CRP in three study groups

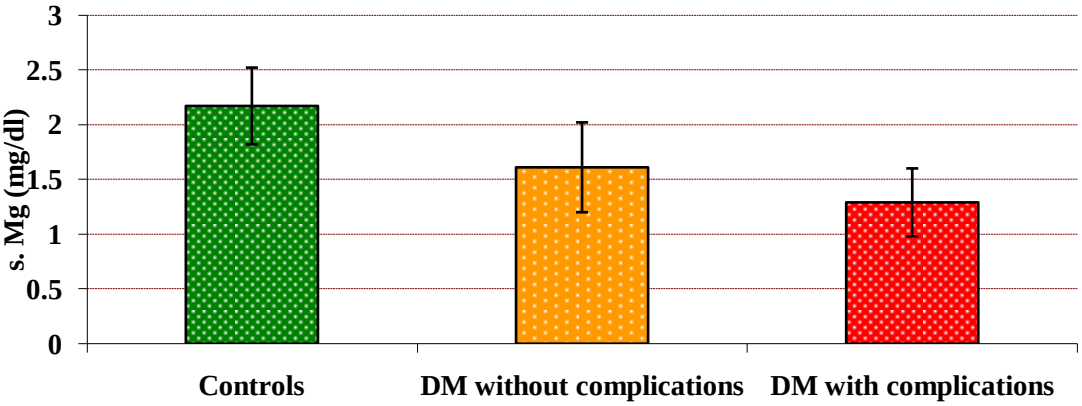

Figure 1b: Sr. Mg in the three study groups

Table 2: FBS and PPBS in the three study groups

Study parameters	Controls	DM without complications	DM with Complications
FBS mg/dl	88.13±18.95	142.97±12.48	187.83±29.89
PPBS mg/dl	127.03±21.42	230.70±26.84	317.00±48.32

Results are presented in Mean ± SD

Study parameters	Controls Vs DM without complications	Controls Vs DM with complications	DM without Complications Vs DM with Complications	Effect size Controls Vs DM without complications	Controls Vs DM with complications
FBS mg/dl	<0.001**	<0.001**	<0.001**	3.37	3.93
PPBS mg/dl	<0.001**	<0.001**	<0.001**	4.21	5.02

p values are obtained by using the Post-hoc Tukey test

Table 3: Blood urea and Sr. Creatinine in the three study groups

Study parameters	Controls	DM without complications	DM with Complications
Bl.Urea mg/dl	24.33±5.84	27.83±8.33	33.57±7.47
Sr.Cr mg/dl	0.90±0.15	0.85±0.21	1.07±0.27

Results are presented in Mean±SD

Study parameters	Controls Vs DM without complications	Controls Vs DM with complications	DM without Complications Vs DM with Complications	Effect size Controls Vs DM without complications	Controls Vs DM with complications
B.Urea mg/dl	0.157	<0.001**	0.009**	0.19	1.36
Sr.Cr mg/dl	0.645	<0.001**	<0.001**	0.08	0.77

p values are obtained by using the Post-hoc Tukey test

Table 4: Lipid parameters in the three study groups

Study parameters	Controls	DM without complications	DM with Complications
TC mg/dl	179.50±44.64	239.23±36.08	267.07±31.89
TG mg/dl	121.47±28.74	186.80±58.17	257.80±71.05
HDL mg/dl	47.87±4.24	35.07±5.56	28.97±6.72
LDL-C mg/dl	103.03±33.95	164.13±37.28	187.60±28.96
VLDL mg/dl	24.40±5.71	37.33±11.60	52.90±14.12

Results are presented in Mean±SD

Study parameters	Controls Vs DM without complications	Controls Vs DM with complications	DM without Complications Vs DM with Complications	Effect size Controls Vs DM without complications	Controls Vs DM with complications
TC mg/dl	<0.001**	<0.001**	0.015*	1.45	2.25
TG mg/dl	<0.001**	<0.001**	<0.001**	1.41	2.48
HDL mg/dl	<0.001**	<0.001**	<0.001**	2.56	3.32
LDL-C mg/dl	<0.001**	<0.001**	0.022*	1.69	2.65
VLDL mg/dl	<0.001**	<0.001**	<0.001**	1.40	2.61

p values are obtained by using the Post-hoc Tukey test

Table 5: Pearson correlation between hs-CRP and Sr. Mg

Group	r value	p value
Controls	-0.421	0.021*
DM without complications	-0.378	0.039*
DM with complications	-0.182	0.335

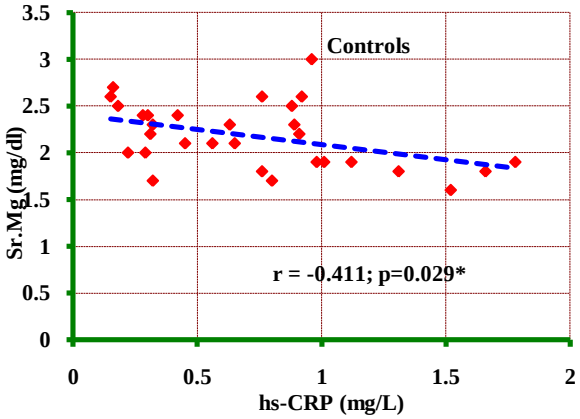

Figure 4a: Scatter plot of hsCRP Vs sr.Mg

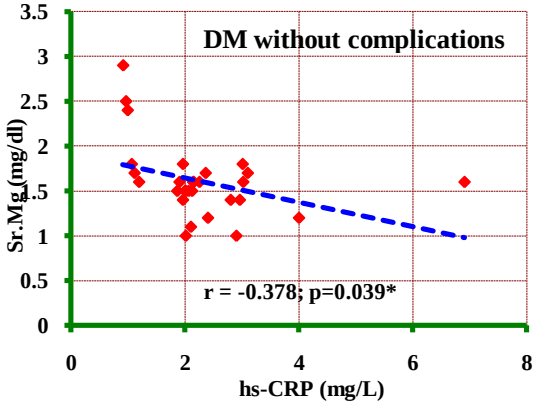

Figure 4b: Scatter plot of hsCRP Vs Sr.Mg

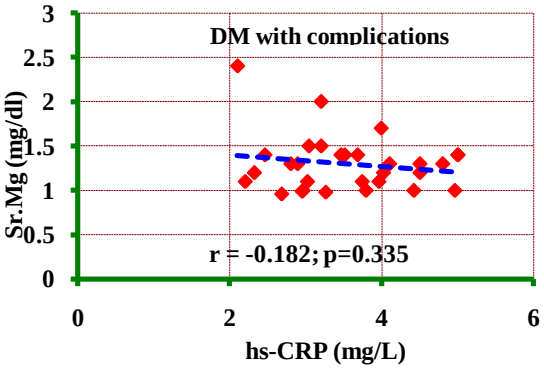

Figure 4c: Scatter plot of hsCRP Vs Sr.Mg

Table 6: Pearson correlation of hs-CRP vs FBS, Total cholesterol, HDL and LDL

Pair	Controls	DM without complications	DM with complications
hs-CRP vs FBS	0.284	0.421	0.682
hs-CRP vs Total cholesterol	0.093	0.446	0.697
hs-CRP vs HDL	-0.035	-0.223	-0.441
hs-CRP vs LDL	0.336	0.368	0.523

Results are presented in r value

Table 7: Pearson correlation of Sr.Mg vs FBS, Total cholesterol, HDL and LDL

Pair	Controls	DM without complications	DM with complications
Sr.Mg vs FBS	-0.122	-0.265	-0.230
Sr.Mg vs Total cholesterol	-0.305	-0.543	-0.295
Sr.Mg vs HDL	0.239	0.261	0.431
Sr.Mg vs LDL	-0.366	-0.341	-0.227

Results are presented in r value

Presently type 2 diabetes has become a leading cause of morbidity and mortality. Prevention of diabetes and its associated burden primarily cardiovascular morbidity and mortality, have become major health issues worldwide. Although insulin resistance and β-cell failure continue to be recognized as the central causal processes in the development of type 2 diabetes, other paradigms have also been evolved .Influenced by the findings indicating an inflammatory basis for cardiovascular diseases and following the "common soil "hypothesis of coronary heart disease and type 2 diabetes, Bruce *et al.,* (2003) have revealed that a low-grade inflammation precedes and predicts, the onset of diabetes in adults.

Malik *et al.,* (2005) studied that high sensitive C-reactive protein (Hs-CRP) independently predicts cardiovascular diseases and whether it can stratify risk in those with diabetes is not well documented.

Brbagallo *et al.,* (2003) shown that magnesium homeostasis has been hypothesized to be a link between insulin resistance, type 2 diabetes, hypertension and cardiovascular diseases.

It is in this background that the current study has been undertaken to assess the serum levels of hs-CRP and magnesium in type 2 diabetic cases without complications and with complications.

Statistically there was no difference between the average age of controls and cases. Immaterial of the sex, cases were primarily selected on the basis of the chronicity of the disease. In the present study, mean body mass index (BMI) was found to be much higher in patients with and without complications compared to controls ($p<0.001$). Similar results were seen in study conducted by Frank Hul *et al., (*2004).

Our study has revealed that hsCRP values are significantly higher in diabetics without (2.27±1.4mg/L) and with complications (3.55±0.86mg/L) as compared to controls (0.72±0.45mg/L). p value < 0.001 in both groups. David *et al.,* (1978) has demonstrated that individuals without inflammation usually have hs-CRP levels below 1mg/L.

Several studies have also suggested that high serum levels of hsCRP are a major contributor to the risk of type 2 diabetes. The hsCRP values are significantly higher in diabetics without complications compared to the controls ($p<0.001$).

Yuji *et al., (*2005), Wang *et al., (*2006), Simin *et al., (*2007) and Abbas Dehghan *et al., (*2007) studies done across the world have projected similar results.

In 1998, a hypothesis was proposed suggesting that long term innate immune system activation, resulting in chronic inflammation, elicited disease instead of repair, leading to the development of type 2 diabetes. Furthermore it is accepted that chronic subclinical inflammation is a part of the insulin resistance syndrome and is strongly related to the features of metabolic syndrome.

The mechanisms by which chronic inflammation can evoke type 2 diabetes are not clear. However it is known that adipose tissue can synthesize and release the main pro-inflammatory cytokines-tumor necrosis factor-alpha (TNF-α), interlelukin-1 (IL-1) and interleukin-6 (IL-6) and that inflammatory markers are associated with body fat mass. Pro-inflammatory cytokines and acute phase reactants are involved in multiple metabolic pathways relevant to insulin resistance, including regulation, reactive oxygen species, lipoprotein lipase action and adipocyte function. Therefore activated innate immunity and inflammation are relevant factors in the pathogenesis of diabetes, with convincing data that type-2 diabetes includes an inflammatory component**.**

In the present study, patients with diabetic complications have higher hsCRP levels compared to cases without complications and these values are far higher than in control group ($p<0.001$). Diabetic complications as suggested by clinical findings correlated with elevated hsCRP levels.

Similar results have been observed in various studies.

Mohan *et al.,* (2005) in their study on 150 subjects selected from Chennai Urban Rural Epidemiology Study (CURES), have similar findings in that the diabetic subjects with CAD had higher CRP levels compared to diabetic subjects without CAD and control subjects.

Minna *et al.,* (2006) in a large cohort study of 1045 cases of diabetes patients aged 45 to 65 years over a 7-year of follow up period, have reported that the mean hsCRP levels were significantly higher in men who died of CHD or who had a fatal or nonfatal myocardial infarction.

With respect to serum magnesium in diabetics, the present study has revealed that the mean serum magnesium level was significantly low in diabetics as compared to control subjects. This indicates the association of hypomagnesaemia with diabetes mellitus. These results are in accordance with the observation of Tosiello *et al.,* (1996), Kao *et al.,* (1999) Chamber *et al.,* (2006) and Diwan *et al.,* (2006), they have all reported a strong association of hypomagnesaemia with diabetes mellitus.

In our study, serum magnesium level in cases with diabetic complications (1.29±0.31) was much lower than those without complications (1.61±0.41). We have also noted that in subjects with diabetic retinopathy serum magnesium levels were much lower when compared to diabetics with other complications. The present finding is in accordance with the studies of Sharma *et al*. They have made a cross-sectional study on 50 diabetic cases with or without complications. Serum magnesium was significantly low in diabetes with complications than without complications ($p<0.001$). Aradhna *et al.,* (2007) shown a strong association with hypomagnesaemia, retinopathy and obesity have been identified. Pham *et al.,* (2007) have also reported that hypomagnesaemia has been linked to poor glycemic control, Coronary artery disease and diabetic microvascular complications. Various studies all over the world have projected similar results.

The precise mechanism for the development of micro vascular changes is not fully understood. It is possible that hypomagnesaemia inhibits prostacyclin receptors function producing an imbalance between prostacyclin and thromboxane effect which has marked atherogenic potential that inturn may lead to microvascular complications. Magnesium deficiency also leads to increased urinary thromboxane levels and enhanced aldosterone-secreting effect of angiotensin-II.

These effects are associated with a decrease in insulin action, suggesting that magnesium deficiency may be a common factor associated with insulin resistance and vascular disease.

We have evaluated the relation of hsCRP with lipid parameters. The serum total cholesterol, Triglycerides, LDL-C and VLDL Cholesterol levels were significantly higher in both the groups of cases compared to controls ($p<0.001$). The mean HDL-C was significantly lower in both the groups of cases ($p<0.001$).

Chapman *et al., (*2006) studied that dyslipidemia has been proved to be one of the major risk factor for CHD. Both VLDL-C and LDL-C are associated with atherogenic process and there is increasing evidence that HDL-C prevents atherogenesis.

There is a positive correlation of hsCRP with FBS ($r =0.421$ w.o.c; $r =0.682$ w.c), Serum total cholesterol ($r=0.446$ w.o.c; $r=0.697$) and LDL-C ($r=0.368$ w.o.c; $r=0.523$ w.c) and negative correlation with serum HDL-C ($r=-0.223$ w.o.c; $r=-0.441$ w.c).

Various studies did have projected similar results. Yuji *et al.,* (2005) in their study have made a comparison of log hsCRP with FBS, Total Cholesterol, Triglycerides and HDL-C. They found a positive correlation of hsCRP with FBS, T-Cholesterol, Triglycerides and negative correlation with HDL-C.

Ridker *et al., (*2003) and Han *et al., (*2002) suggestd that hsCRP level is known to be significantly related to the presence of the metabolic syndrome and also significantly correlated with levels of obesity, hyperglycemia, hyperlipidemia and hypertension.

There are many possible mechanisms by which hsCRP enhance atherosclerosis- hsCRP activates the complement pathway and induces adhesion molecule expression by human endothelial cells. hsCRP is also known to play a role in monocyte recruitment into the arterial wall. hsCRP enhances the entry of LDL particles into macrophages and it has been found to induce plasminogen activator inhibitor-1

expression. Elevated CRP also stimulates endothelial production of E-Selectin, ICAM-1 and VCAM-1, important mediators of impaired vascular reactivity, reduced insulin delivery and increased peripheral insulin resistance, Thus, the positive association between CRP and type 2 diabetes may simply reflect underlying endothelial dysfunction and subclinical atherosclerosis.

We have also evaluated the relation of serum magnesium with lipid parameters. There is a negative correlation of serum magnesium with total Cholesterol (r=-0.543 w.o.c; r=-0.295 w.c) and LDL-Cholesterol, (r=-0.341 w.o.c; r=-0.227 w.c) and positive correlation with HDL-C (r =0.261 w.o.c; r=0.431 w.c).These findings are in agreement with other studies. Nasri *et al., (*2006), in their study on 122 patient with diabetes, has observed an inverse correlation of serum.Mg with serum total Cholesterol and LDL-C and a non significant correlation of serum. Mg with HDL-C.

In another study, Fernando *et al.,* have observed that subjects with type 2 diabetes had higher levels of serum total cholesterol, LDL- C and Triglycerides, and lower levels of HDL-C values than healthy subjects. Fernando *et al., (*2000) was also observed that hypomagnesaemia is linked to low serum HDL-C, irrespective of serum glucose values.

In present study, the raise in FBS and PPBS are statistically significant ($p<0.001$) in diabetic cases with and without complications when compared to the controls. This observation is in agreement with other studies. Studies on 200 Indian diabetic subjects have shown both micro and macrovascular complications are mainly dependent on glycemic control rather than duration of diabetes.

Kareem *et al.,* (2004) in their study on 70 diabetic patients without or with complication, have shown that FBS and PPBS values were found to be significantly higher. Studies have in fact proved that PPBS is an independent risk factor for cardiovascular disease in type 2 DM, irrespective of FBS and HbA_{1C} levels. In the present study an attempt has been made to assess the relationship between hsCRP and serum. Mg. There is a negative correlation between hsCRP and serum. Mg (r=-0.378 w.o.c; r=-0.182 w.c) in both the groups of cases and controls (r=-0.421

Mechanisms relating to low magnesium levels and elevated CRP values might be linked to oxidative stress or endothelial dysfunction. Magnesium deficiency is known to inhibit endothelial growth and migration, stimulates the synthesis of nitric oxide and some inflammatory markers in vivo, thus directly modulating microvascular functions.

Since elevated levels of hs-CRP and lowered levels of magnesium in serum correlative positively with altered lipid profile, these changes seem to be related to the degree of severity of the complications and hence may be useful as markers of complications in type 2 diabetes.

Hence further studies on serum hs-CRP and magnesium to prevent the complications of diabetes will be interesting and helpful.

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