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Association of elevated levels of serum CRP with development of cardiovascular disease in known cases of type II DM- An Observational case control study

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Running Title:-Association of CRP, CVD in type II DM

Abstract

Introduction

Diabetes Mellitus is directly linked with the development of cardiovascular diseases, but there is very little documented information available regarding its association with C-reactive protein (CRP).

Aim

To analyze the association between CRP and cardiovascular diseases (CVD) in Type II Diabetes Mellitus patients.

Material and Methods

This was a case control observational study, carried out among 200 participant subjects (100 controls & 100 cases). Blood sample (5 ml) was collected from each subject and Serum was separated by centrifuging blood at 2000 rpm for 15 min, Estimation of CRP were done on semi-autoanalyzer (Robonikreadwell touch) in clinical biochemistry laboratory, lipid profile and other routine investigations by autoanalyser. All the statistical analysis was done by using the Windows based SPSS statistical package (Version 23.0) Student's t-test and Pearson correlation analysis was applied and p-values <0.05 were taken as the significant.

Results

The mean value of CRP in the subjects with type II Diabetes Mellitus was 3.81 mg/L and in control was 2.20 mg/L ($P<0.05$). The weight and waist circumference, were also significantly different ($P<0.05$) between the study groups CRP had an insignificant correlation with the BMI ($r= 0.15, p>0.05$) as well as with waist circumference ($r=0.18, p>0.05$). Also significant correlation was found in between the HbA1c (%) & Blood glucose (F) (mg/dl).

Conclusions

The present study finding suggests that deranged blood sugar & HbA1c levels regulate the level of CRP that could lead to complication like CVD. Also, there is a strong degree of association between type II DM and CRP in the study.

Key Words: Type II DM, CRP , lipid profile, CVD, HbA1c

Introduction

CRP is a sensitive inflammatory marker, and it is one of the biomarkers that have been most extensively studied for CVD [1].

The increased levels of CRP contribute to the recognition of subjects who are at risk of developing CVD [2-5].

It is well documented that chronic inflammatory changes are evidenced by elevated hsCRP might be a cause of underlying aetiology and manifestations of TIIDM [6,7]

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TIIDM shares various inflammatory mechanisms with cardiovascular events. This may have ambiguous implications for the choice of therapeutic prognosis in the disease. Although the exact mechanisms are not well understood still and documented [8,9].

Different cohort studies have established the correlation between the elevated level of CRP and CVD risk in subjects either with well-established disease i.e CVD as well as in its development. [1,4,8,10].

It makes hsCRP valuable in the well-established cases of type II Diabetes Mellitus as a test to estimate progression of the disease and its prognosis and also to establish the risk for the development of CVD in Diabetic patient.

A scientific statement documented by both Centre for Disease Control (CDC) and American Heart Association (AHA) that CRP is the only sensitive inflammatory marker that can be used for risk prediction of CVD [11].

Various studies have documented the association between CRP and CVD[8,10], but the data on the population residing in Southern M.P region is very limited on the association of CRP, type II Diabetes Mellitus and CVD. So the present study was planned to find the correlation of CRP, type II Diabetes Mellitus and CVD in the population of Southern M.P region.

Materials and Methods

Study Participants

This was a case control observational study carried out in the Department of Biochemistry, Central Research lab and Central clinical Lab of LNCT Medical & Jk Hospital Bhopal. On sample size of 200 participants (100 cases & 100 control)[Since it is a generalized rule, sample size of around 200 subjects provide an margin of error which is acceptable i.e between 4 to 8%]. Over a period of 20 months from November 2020 to July 2022 after taking the informed consent from the study participants. The institutional ethics committee (IEC) approved the study [letter no. LNCT/Ph.D/2020/BC/018].

Inclusion Criteria (Cases)

(a)All patients attending Medicine OPD having signs and symptoms of CVD & type II DM

(b) Patient having dyslipidaemia

Inclusion Criteria (Control)

(a)All the patient attending medicine OPD with no sign and symptoms of CVD

(c) Study participants between the age group of 20 and 60 years.

Exclusion Criteria

(a) Smokers

(b) Subjects with any chronic disease/ acute infection.

(C) Patients with H/O drugs (effecting CVD) intake 1 month prior to sampling

Data Collection

Information about the subject's age, sex, monthly income, lifestyle, family history of diabetes mellitus and other chronic disorders were recorded. Anthropometric measurements like height, weight and waist circumferences were also measured. when the individuals were dressed in light clothes and were not wearing shoes. A plastic non stretchable tape used to measure the hips circumference. The minimal horizontal girth between the costal margins and the iliac crests at the end of normal expiration was used to calculate waist circumference. Body mass index (BMI) was calculated as by dividing weight in kg by the square of height in meters [12].

Assay Methods

Blood sample (5 ml) was collected from each subject. Serum was separated by centrifuging blood at 3000rpm for 10 min, Estimation of CRP were done on semi-auto analyzer (Robonikreadwell touch) in clinical biochemistry laboratory, Lipid profile was estimated- Total Cholesterol (TC), Triglyceride (TAG) by enzymatic method and High-Density Lipoprotein (HDL) measured by non HDL precipitation method followed by enzymatic method in serum samples [13]. The low-density lipoprotein (LDL) cholesterol concentration was calculated by friedwald's formula using (TC, HDL, TAG) (LDL cholesterol= TC-HDL-TAG/5 (mg/dL) [14] by Vitros-250 auto analyser Johnson & Johnson, USA.

A turbidimetric immunoassay for the determination of CRP in serum was used. Measuring range is 0.15 mg/L to 5 mg/L [15].

The study followed the risk stratification as recommended by American Heart Association (AHA) [15]:

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Low risk: <1.0 mg/L
Average risk: 1.0 to 3.0 mg/L

High risk: >3.0 mg/L

Statistical Analysis

Student's t-test and Pearson correlation analysis was applied and p-values <0.05 were taken as the significant , All the analysis was done by using the Windows based SPSS statistical Package (Version 23.0) and P- values <0.05 were taken as the significant.

Result

As compare to control subjects, the type II DM cases in the study were older (P <0.0001) and have higher body Mass Index (BMI) (P <0.0001). They also had higher blood glucose (Fasting) and HbA1c levels (P <0.0001). The mean value of CRP in the subjects with type II DM (cases) was 3.81 mg/L and in control subjects were 2.20 mg/L, the CRP levels were higher among the cases (P <0.0001), WC were also find significant statistically (P<0.05) between the study groups [Table/Fig-1].

Parameters	**cases (N = 100)**	**Control (N = 100)**	**P value**	**t test**
Age (Yrs.)	49.60±9.9	43.66±9.3	<0.0001*	4.37
Height (cm)	148.05±14.1	150.09±15.0	0.32	0.99
Weight (kg)	72.88±12.7	65.88±12.94	0.0002	3.86
Body mass Index (kg/m²)	33.11±7.41	28.19±8.04	<0.0001*	4.50
WC(Inches)	103.49±9.37	91.47±8.64	<0.0001*	9.43
HbA1c (%)	8.61±2.24	5.33 ± 0.98	<0.0001*	11.57
Blood glucose (F) (mg/dl)	232.66±55.78	91.56 ± 8.84	<0.0001*	24.98
CRP(mg/L)	3.81±0.91	2.20±0.96	<0.0001*	12.17

Table/Fig 1. Comparison of Anthropometrics & Blood Glucose Parameters

P <0.05 considered significant; *: Significant; WC: Waist circumference

	Parameters	**r value**	**P value**
hsCRP Verses Anthropometric measurement & Blood glucose	**Age (Yrs.)**	0.02	0.71
	Height (cm)	-0.13	0.15
	Weight(kg)	0.02	0.92
	Body Mass Index (kg/m²)	0.14	0.16
	WC(Inches)	0.19	0.16

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Parameters	**Blood glucose (F) (mg/dl)**	0.14	0.12
	HbA1c (%)	0.19	0.15

Table/Fig-2. Correlation of serum CRP with the anthropometric measurement & Diabetic panel in type II DM patients

P Value < 0.05considered significant

The corelation between CRP and the anthropometric measurements i.e BMI & WC) indicates a P value of >0.05.Similar insignificant association was found in between the CRP and the blood glucose parameters (Blood Glucose (F) & HbA1c) [Table/Fig-2]

	Parameters	**r value**	**P value**
CRP Verses Cardiovascular risk factors	**Total Cholesterol (mg/dl)**	0.14	0.03*
	TG(mg/dl)	0.09	0.41
	HDL-C(mg/dl)	0.20	0.04*
	LDL-C(mg/dl)	0.13	0.27
	VLDL-C(mg/dl)	0.07	0.50

[Table/Fig-3]: Correlation of serum CRP with the lipid profile as a cardiovascular risk factors in patients of type II DM.

Correlation coefficient (r); *: Significance; TC: Total Cholesterol, TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein

We have found a positive and significant correlation between hsCRP Vs TC and HDL-C [Table/Fig-3].

Discussion

Diabetes Mellitus is the condition of increased level of blood glucose, if for a long term blood glucose remain deranged in any subject it leads to the complications such as diabetic retinopathy ,diabetic nephropathy, diabetic neuropathy and diabetic ulcer foot.

Diabetes mellitus is the most prevalent endocrine disorder and the disease burden related to this cluster of disorders is commonly associated with CVD [16,17].

Festa et al. demonstrated that patients who have DM had higher CRP levels as compare to other subjects that do not have DM [18]. Likely results are also came out in the study also that CRP is positively correlated with DM.

Another study also reported that people with high CRP level are diagnosed with DM [19]. Since our study also concluded that CRP is also associated with DM are the similar findings of the study. A meta-analysis demonstrated that high CRP levels associated with T2DM in near future diagnosed based on the basis of blood glucose (F) as one of the parameter [20]. Which is also in accordance with our study. All these studies findings support that chronic low-grade inflammation may be involved in the development of DM.

In another study, researchers found a positive association in between CRP levels and diabetic parameters [21]. we have also find the similar findings in our study.

In a study conducted by Hyemin Jeong et al on a total of around 5887 participants showed that CRP level was associated with an increased risk of dyslipidaemia & diabetes mellitus[22], which is in accordance to our study that also shows the similar results

In a study conducted by N Pannacciulli et al concluded that CRP was positively correlated with age, body mass index (BMI), waist, fasting glucose [23], similar results are also found in our study which stated that age, body mass index (BMI), waist circumference, fasting glucose are positively significant with CRP

In a study by Paul M Ridker et al shows that CRP levels is a useful biomarker of risk for CVD and diabetes mellitus [24]. In our study we have also find the similar results i.e the positive association of CRP , CVD & DM

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Another study concluded that higher levels of CRP have a positive correlation with type 2 DM [25] we have also encountered the similar results in the study which shows that there are higher levels of crp in the patients of typeIIDM.

Conclusion

We conclude that CRP levels were significantly higher in patients (cases) with type II DM having deranged blood sugar level when compared to the healthy controls. A significant correlation was found in the study between risk factors of CVD (Lipid Profile, blood sugar panel and CRP levels). We have to see with good glycemic control with therapeutic agents/Diet modification CRP levels may decrease and to correlates it with dyslipidaemia.

This study needs to be analyzed in a larger group of population of type 2 diabetis mellitis to evaluate screening and early detection of the increased level of CRP in the T2DM subjects helps clinicians to prevent further complications and outcomes in the type IIDM diseased subjects. Therefore, by screening of high-risk individuals the pre diabetic subjects by lifestyle/diet modification changes is an preventive measure to reduce diabetes mellitus -related co-morbidity.

Further study is needed in a large group population of type II diabeties mellitus to test & evaluate our recommendations.

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