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Original Article

High sensitive C-reactive protein, Insulin resistance and Coronary artery disease risk.

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ABSTRACT

Inflammation and insulin resistance (IR) are forerunners of cardiovascular disease (CVD) and diabetes .Asians have an underlying pro inflammatory state that may contribute to their increased risk for CVD and type 2 diabetes.This study was aimed at proving high sensitive C-reactive protein (hsCRP) ,IR as risk factors for CVD, hs CRP is also associated with metabolic syndrome. 133 subjects of mean age 48 yrs, both males & females attending cardiology department for master health check up were included in this study. Anthropometric measures (height, weight, body mass index(BMI), waist circumference(WC), blood pressure), biochemical parameters(fasting plasma glucose(FG), serum total cholesterol (TC), triglycerides(TGL), High density lipoprotein cholesterol(HDLc), fasting insulin , hs CRP) were measured in all subjects. Insulin resistance is measured by Homeostasis model for assement of insulin resistance(HOMAIR).Based on hs CRP two groups were divided-group1 with CRP <3 mg/L, group2 with hs CRP > 3 mg/L . All the parameters were compared between the groups, correlation was also observed. Statistical analysis used: Pearson's correlation coefficients were used to assess the associations, student's t test, and multivariate binary logistic regression analysis was used to compare the groups p value is significant for HDLc($p<0.008$) fasting glucose (FG)($p=0.035$), HOMA IR($p<0.04$) .BMI is positively related with hs CRP, the mean hs CRP levels were elevated with increasing number of metabolic syndrome components. Gender specific analysis showed that HDLc, HOMAIR were significant predictors of inflammation in males but not in females. According to this study , variables like HDLc, HOMAIR were significant predictors of inflammation . Inflammation is associated with metabolic syndrome . Hence ,the role of inflammation in CVD can provide new opportunities for diagnosis& treatment.

1. Introduction

Pro-inflammatory state is associated with increased risk for cardiovascular disease (CVD) and diabetes mellitus[1]. During inflammation, elevation of acute phase C- reactive protein(CRP) is evident[2,3]. Enhanced CRP expression is seen in IR[4]. American Heart Association and Center for Disease Control and Prevention[5] stated that, in healthy men and women , the levels of hs CRP <1,1-3,>3 mg/L distinguish between low, moderate, high risk for future CVD risk[6].

Several studies had proven hsCRP as a significant predictor of CVD & diabetes risk.[7] The purpose of this study is to focus on the significance of hsCRP by comparing the traditional risk factors , HOMAIR in groups 1 and 2 , to observe the association of hsCRP with BMI and with individual components of insulin resistance syndrome.

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2. Materials and Methods :

This study was conducted over a period of one year. It was a cross sectional observational study. The study included 133 subjects -both males and females. Subjects attending the department of cardiology, for master health check up without any previous history of acute illness, infections, post coronary artery bypass graft (or) electrocardiograph evidence of heart disease were included in the present analysis. This study was approved by institutional ethical committee. Informed consent was taken and a questionnaire was prepared to elicit the history of smoking, alcohol intake, family history of diabetes, CVD, stroke, drug history which included intake of statins and aspirin. Subjects on statins / aspirin were excluded from analysis as they would alter the insulin resistance; female subjects with polycystic ovarian disease were also excluded.

In the central research lab, fasting and post prandial blood samples were collected from all the subjects for estimation of Glucose, lipid profile(total cholesterol(TC),triglycerides(TGL), high density lipoprotein cholesterol (HDLc), Insulin, hsCRP. Anthropometric measures (height, weight, BMI, waist circumference were taken from all the subjects. Out of 133 subjects, 29 were diagnosed for the first time as diabetics based on World Health Organisation criteria. All 133 subjects were divided into 2 groups, Group 1 with hsCRP <3mg/L and Group 2 with hsCRP >3mg/L. Biochemical and anthropometric measures were compared between the two groups. Gender specific analysis for females & males was also done.

Measuring Insulin resistance:

IR was calculated by using the formula based on fasting plasma glucose (mg/dl) & fasting serum insulin (mu/L).

$$\text{HOMA IR} = \frac{\text{FPG (mg/dl) x FI (mu/L)}}{405}$$

3. Results :

SPSS12.0 software (SPSS Inc., Chicago, II, USA) was used for statistical analysis. Continuous variables were expressed as mean values ± SEM. Pearson's correlation coefficients were used to assess the associations between the continuous variables. Differences between the groups of study subjects (based on hsCRP) were examined using student's t-test. Binary logistic regression analysis was performed with study group (based on hsCRP) as dependent variable and other significantly associated variables as independent variables. A p value <0.05 was considered statistically significant.General profiles of the subjects in terms of the study groups they belong to was described in table1. HDL cholesterol in the subjects with hsCRP > 3 mg/L was significantly lower than the group of subjects with hsCRP < 3mg/L. On the contrary, the level of HOMA IR and fasting blood glucose was significantly higher in subjects with hsCRP > 3 mg/L as compared to their counterparts. The results of multivariable logistic regression analysis showed that after adjusting for confounding, HDL cholesterol ($p < 0.008$) and HOMA IR ($p < 0.04$) were significant predictors of inflammatory state(table 2). However gender- segregated multivariable analysis revealed that among males (table:3) the overall negelkeker R square value increased from 0.145 to 0.286 indicating lesser variability among the study subjects. Variables like HDLc ($p<0.003$), HOMAIR ($p=0.012$) were significant predictors of inflammation. Similar findings were not seen among females with a much lower negelkeker R square value (0.073). Fig.1 showing the positive correlation of log CRP with BMI, Fig.2 explains increasing levels of mean hs CRP with increasing number of metabolic syndrome components.

Table: 1 comparison of parameters in group 1and group2.

Parameters	Group 1 n=60 Mean ±SEM	Group 2 n=73 Mean ±SEM	t test	P value
AGE (yrs)	47.25 ± 1.417	47.83 ±1.077	-0.335	0.739
TC(mg/dl)	193.88 ± 5.94	188.04 ± 5.06	0.753	0.453
TGL(mg/dl)	136.98±9.60	136.35±6.91	0.54	0.957
HDL(mg/dl)	44.31± 1.64	40.20±1.06	2.162	0.032*
Waist(cm)	91.5±1.68	91.39±1.09	0.053	0.958
BMI(kg/m2)	26.06±0.54	26.58±0.48	-0.724	0.471
SBP(mmHg)	132.66±1.34	134.49±1.33	-0.432	0.667
DBP(mmHg)	82.3±0.91	86.13±0.80	-1.489	0.139
FG(mg/dl)	99.83±2.42	110.1±3.89	-2.129	0.035*
Insulin(mU/L)	9.55±0.93	10.7±0.67	-1.018	0.311
HOMA IR	2.31±0.2	2.9±0.20	-2.076	0.040*

a)P* <0.05 (significant) . b)n= number of subjects, c) SEM (standard error of mean)

Table 2; Multivariable binary logistic regression analysis of the confounding variables

		B	S.E.	Wald	Df	Sig.	Exp(B)	95.0% C.I.for Exp(B) Lower	Upper
Step 1(a)	HDL	-.050	.019	7.124	1	.008	.951	.917	.987
	FG	.013	.009	2.118	1	.146	1.013	.995	1.031
	HOMAIR	.264	.128	4.232	1	.040	1.302	1.013	1.675
	Constant	.240	1.136	.045	1	.833	1.271		

a) Nagelkerke R square 0.145

Table 3; Gender Specific Multivariable binary logistic regression analysis of the confounding variables

		B	S.E.	Wald	Df	Sig.	Exp(B)	95.0% C.I.for Exp(B) Lower	Upper
Male	HDL	-.102	.034	9.128	1	.003	.903	.846	.965
	FG	.013	.015	.836	1	.361	1.013	.985	1.043
	HOMAIR	.557	.222	6.301	1	.012	1.745	1.130	2.696
	Constant	1.541	1.740	.784	1	.376	4.669		
Females	HDL	-.015	.028	.297	1	.585	.985	.933	1.040
	FG	.014	.012	1.389	1	.239	1.014	.991	1.038
	HOMAIR	.105	.185	.326	1	.568	1.111	.774	1.595
	Constant	-.782	1.703	.211	1	.646	.458		

a) Nagelkerke R Square for males = .286 and b) for females = .073

Figure: 1 correlation of BMI with log hs CRP

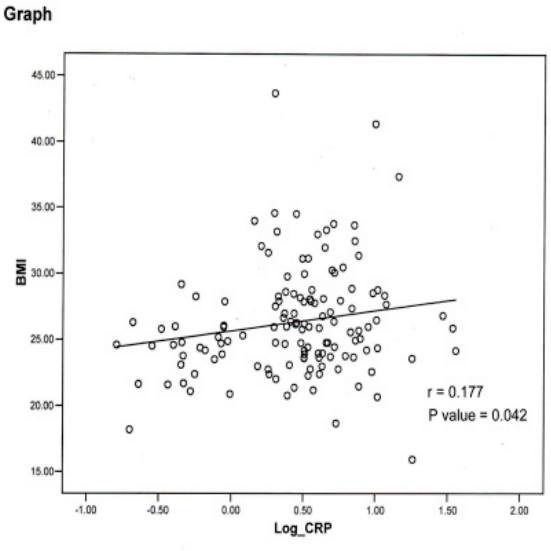

Figure: 2 , Mean hs CRP with increasing number of metabolic syndrome components

Components of Metabolic Syndrome (Modified N C E P ATP III Guidelines)

Fasting glucose > 100mg/dl, TGL >150mg/dl, Low HDL(<40mg/dl in males, <50mg/dl in females, Hypertension ≥ 135/85 mm Hg / medication, Waist circumference > 80cm (females), >90cm (males)

4. Discussion:

The role of inflammation has become well established over the past decade. Large population based studies such as MONICA[8], the Atherosclerotic risk in communities study[9] ,the NHANES[10] , has compared persons in the lower tertile of hsCRP with those in the upper tertiles, showing that major coronary events were observed for the upper tertile with lower tertile used as a reference. Data from Indian Atherosclerotic Research Study (IARS) stated that routine measurement of hsCRP will be useful in prediction of CVD risk in persons of south Asia who were particularly high risk for type 2 DM and CVD[11,12].

With this background, the present study was carried out in south Indian subjects(n = 133) to bring out the importance of inflammation and insulin resistance as cardiovascular disease risk markers. Inflammation involved in atherosclerosis is elicited by many other risk factors like cigarette smoking, insulin resistance / diabetes and essential hypertension[13]. Pro-inflammatory state releases one of the important cytokines- tumor necrosis factor- α(TNF-α), which activates a variety of serine protein – kinases. These activated protein kinases increase serine phosphorylation of Insulin Receptor Substate – 1 and 2. This will lead to decreased activity of Phosphotidylinositol3 kinase. Hence the down stream effects of insulin signaling are inhibited leading to insulin resistance. Thus pro-inflammatory cytokines contribute to insulin resistance[14]. Hence the combination of an active, ongoing inflammatory process and presence of IR might act synergistically in promoting coronary risk[15].

Yudkin et al [16] showed that CRP levels were associated strongly with IR , calculated from HOMAIR. But there are very few studies in south Indians observing the association[17]. This study could be one of the few which highlight the role of inflammation in insulin resistance. Insulin resistance will cause hyperglycemia. This is mainly due to decreased translocation of glucose transporter GLUT4 to the membrane for uptake of glucose by the cells. Hyperglycemia will increase the production of Reactive Oxygen Species, Advanced Glycation End products (AGE) and Sorbitol. AGE increases oxidative stress by activation of NADPH oxidase through specific receptors for AGE(RAGES). RAGE stimulates proinflammtory signaling, leading to activation of NFkβ(Nuclear factor)[18].

Indeed many cardiovascular events occur in individuals with plasma cholesterol and low density lipoprotein cholesterol levels below National Cholesterol Education and treatment Panel threshold. This has enlightened the involvement of inflammation in atherosclerosis and discovery of inflammatory biomarkers for CVD risk prediction. During acute phase reaction(APR) plasma TGL increases and HDLc concentration decreases. Festa et al from IRAS[19] showed that CRP positively correlated with BMI, TGL and inversely with HDLc. The association was proved in present analysis also. But there is no significant difference in TGL levels in between the study groups. This may be due to relatively small sample size , TGL is not normally distributed in this study group. Where as HDLc showed statistical significance. This could be explained by the role of APR on HDLc metabolism. Acute phase HDL is characteristically decreased of apoAI content.SAA(serum amyloid A), an acute phase response protein becomes the major apoprotein of HDL during inflammation[20,21]. In APR, HDLc levels decrease in the circulation due to remodeling of HDL by an acute phase protein group II a secretory phospholipase A2(SPLAr – IIa), which increases HDL catabolism[22].

Obesity exhibits a distinct effect on inflammation. But how and when obesity might initiate an inflammatory response remains incompletely understood. The increase

in adipose tissue contributes to proinflammatory mileu and inflammatory adipokines may promote insulin resistance, endothelial dysfunction and finally atherosclerosis. The inverse association between high levels of physical activity or exercise training and markers of chronic inflammation such as hs CRP has been consistently reported[23]. Several previous studies had reported stronger correlation between BMIandCRP[24].

Metabolic syndrome(MS) - constellation of cardiometabolic disease risk factors has become a global epidemic[25]. Inflammation may be an important etiology of MS . Chemokines secreted from the adipocytes attract the macrophages around the dying tissue. These macrophages release cytokines (TNF-α &IL-6) that further activate inflammatory response and worsen insulin resistance. IR at the level of adipocytes leads to dyslipidemia and hyperglycemia which may further progress to MS . The III NHANES, showed that subjects with MS were more likely to have elevated levels of markers of inflammation[26]. In the present analysis also, it was clearly demonstrated that increasing number of compounds of IRS (FG, HDL, TGL, WC, hypertension) paralleled increasing levels of mean hs CRP. Thus this study reiterates the role of hs CRP in MS in south Indian subjects.

But the limitations of our study were 1. Descriptive study 2. Relatively small number of subjects 3. Can not estimate the future risk. 4.Association proven ,longitudinal studies are required.5.History on Hormone replacement therapy / Oral contraceptive usage in case of females was not taken, which may influence hs CRP levels.

The important findings of our study 1. Mean hs CRP of the study subjects was relatively high, this may be due to ethnicity , south Indians will have high waist to hip ratio and abdominal obesity which will lead to adipocytokine production. 2. Inflammation & insulin resistance were closely associated. 3. hs CRP has a great influence on metabolic syndrome.

Hence by this study it is concluded that HDLc , HOMAIR were significant predictors of inflammation. hs CRP is also associated with metabolic syndrome which is again considered a risk factor for CVD. Hence new knowledge about inflammation in CVD and insulin resistance will provide new opportunities for diagnosis and prediction of CVD risk. This may lead to new treatments for this life threatening disease.

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