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Role of inflammatory markers hsCRP and gamma interferon in metabolic syndrome

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ABSTRACT

The number of healthcare institutions are increasing steadily in Nepal, but the issues related to biomedical waste generation still remains. These wastes pose a major problem because of its infectious potential. In addition, wastes that are generated in a dental setup also causes environmental and health issues. The knowledge of biomedical waste management and its effective disposal goes a long way in reducing the overall burden of infectious diseases among the general population and the healthcare professionals alike. Questionnaires regarding knowledge and awareness of biomedical wastes helps in determining the current status regarding its management. This study was hence designed to create a translated version of a questionnaire in Nepalese language to determine the awareness and knowledge among the dental students, dental auxiliaries, foremen and helpers in a dental college in Eastern Nepal.

KEYWORDS : Metabolic Syndrome, hsCRP, Gamma interferon, Inflammatory markers

INTRODUCTION

A relatively high prevalence of the MS is a worldwide phenomenon. In India, prevalence is relatively high. The clustering of risk factors that constitute the MS is found to be common in most countries of the world. In the Americas, in Europe, and in India, at least one-fourth of the adults carry the syndrome [1].

Clinically, the serum biomarkers like IL-1, IL-6, IL-18, TNF, CRP, IFN gamma are readily measured and alteration in these biomarkers may predict development of MS. Several pro-inflammatory cytokines have been shown to be elevated in parallel with an increasing number of components of the syndrome, whereas the anti-inflammatory and adipocyte-specific substance adiponectins consistently lower [2-5]. Obesity, IR and type 2 DM have been characterized as chronic inflammatory states that are associated with abnormal concentrations of cytokines, acute-phase reactants and other inflammatory signalling markers [6-10]. Among them, CRP is strongly associated with Insulin resistance or metabolic syndrome [11-15]. Yuji Tajiri et al studied the relationship between hs-CRP in Japanese patients with type 2 DM. They found that hs-CRP was significantly related to the presence of MS and also significantly correlated with levels of obesity, hyperlipidemia and hyperglycemia. This data suggest that inflammation is strongly related to all components of the MS in subjects with diabetes [16]. Another population study from Japan [17], showed a statistically significant positive correlation between CRP, BMI, triglycerides, LDL, fasting glucose, fasting insulin, uric acid, and negative correlation with HDL. Levels of CRP increased with increase in the number of components of the MS.

Increased expression of proinflammatory Th1 cytokine IFN-gamma is also seen in cases of IR and obese patient of MS [18]. So, identifying these markers by a single blood test well before a disease begins will help to improve mechanistic understanding of IR [19].Keeping all these observations from different reports, the present study was designed to study role of highly sensitive markers of proinflammatory conditions like hs-CRP and IFN gamma in MS in our study area.

MATERIALS AND METHODS

The present study was undertaken as a cross sectional observational study in a tertiary care hospital in the department of Biochemistry in association with department of Medicine**.** During study period, 49 subjects having BMI more than 25 but not suffering from MS were selected as control subjects following screening for exclusion and inclusion criteria. On the other hand, 71 patients having BMI greater than 25 and meeting the criteria for diagnosis of MS following NCEP guidelines were selected as case group after meeting the requisite inclusion and exclusion criteria.

Inclusion criteria- Any three of the following five conditions-

(1) Blood pressure more than or equals to 130/85 mm of Hg
(2) Fasting blood glucose more than or equals to 110 mg/dl
(3) Waist circumference: In men more than or equals to 40 inches
In women more than or equals to 35 inches
(4) HDL: Men- less than 40 mg/dl
Women- less than 50 mg/dl
(5) Serum triglycerides - 150 mg/dl or more

The above criteria were according to National Cholesterol Education Programme (NCEP) [20]

Exclusion criteria

(1) Subjects with any hormonal disorder other than DM
(2) Neonate and immunocompromised
(3) History of alcoholism or hepatitis
(4) Liver function test abnormality

12 hours overnight fasting venous blood samples were collected from cases and controls and the samples were centrifuged for the estimation of fasting blood glucose, serum triglycerides, serum total cholesterol, HDL, hs-CRP, gamma IFN. All the biochemical investigations were carried out on a semi automated chemistry analyzer (ERBA) using standard kits. (CREST BIOSYSTEM, CORAL). Estimation of hs-CRP was done by immunoturbidimetry [21, 22] (ERBA DIAGNOSTICS) method in semiauto analyser and gamma IFN by ELISA kit.

RESULTS

Firstly, distribution of age and sex, between case and control group is shown through following tables and figures:

Table 1A: Distribution of male and females in both cases and control groups

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.296[a]	1	.130		
Continuity Correction[b]	1.719	1	.190		
Likelihood Ratio	2.274	1	.132		
Fisher's Exact Test				.155	.095
N of Valid Cases	120				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 14.29.
b. Computed only for a 2x2 table

In the Table 1A, distribution of gender in both case and control groups are shown. It is evident from the data that there is no significant difference between the case and control groups as far as distribution of males and females are concerned. Pearson Chi-Square value is 2.296 and p value is 0.155 which is not significant. The vivid illustration is shown in figure no 1A and 1B.

Table 1B: Mann-Whitney test to analyze the significance of difference between age in case and control group:

	Case group mean rank	Control group mean rank	Case group (median)	Control group (median)	Mann-Whitney U	Z value	p value asymp. Sig. (two tailed)
Age in year	64.65	54.48	49	45	1444.500	-1.577	0.115

In this table no 2, significance of difference between age in case and control group is analysed. Age distribution shows no significant difference between the two groups. So it can be said this study is age matched.

Difference between the central tendencies of the study parameters between the case and control groups are shown in the following tables:

Table1C: Non parametric assay for analysing of parameters between case and control group: (Case group = 1, Control group = 0).

Ranks				
	Grouping	N	Mean Rank	Sum of Ranks
Age in yrs	.00	49	54.5	2669
	1.00	71	64.6	4590
	Total	120		
Serum TG in mg/dl	.00	49	27.00	1323.00
	1.00	71	83.62	5937.00
	Total	120		
Serum HDL in mg/dl	.00	49	82.16	4026.00
	1.00	71	45.55	3234.00
	Total	120		
HOMA-IR	.00	49	24.51	1152.00
	1.00	71	82.66	5869.00
	Total	120		
FBG (mg/dl)	.00	49	24.91	1171.00
	1.00	71	82.39	5850.00
	Total	120		
hs-CRP (mg/l)	.00	49	40.54	1905.50
	1.00	71	72.05	5115.50
	Total	120		
Gamma IFN (pg/ml)	.00	49	48.40	2275.00
	1.00	71	66.85	4746.00
	Total	120		
WC (cm)	.00	49	31.62	1486.00
	1.00	71	77.96	5535.00
	Total	120		

In the Table 1C, distribution of different test parameters are shown. Group 1 and group 0 indicated the case and control subjects respectively. Differences between the mean rank values of the parameters suggest a significant difference in the distribution of study parameters except their ages, between the case and control population. The results of the Table 1 were validated by the Mann-Whitney test for determination of the significance of difference in Table 2.

Table 2: Mann-Whitney test to analyze the significance of difference between the study parameters in case and control group:

	Case group mean rank	Control group mean rank	Case group (median)	Control group (median)	Mann-Whitney U	Z value	p value asymp. Sig. (two tailed)
Serum hs-CRP in mg/L	72.05	40.54	2.31	1.00	777.500	-4.900	<0.001
Serum gamma interferon in pg/ml	66.85	48.40	9.79	7.98	1147.000	-2.867	.004
Plasma FBG in mg/dl	82.39	24.91	162	88	43.000	-8.936	<0.001
HOMA-IR	82.66	24.51	2.40	0.70	24.000	-9.042	<0.001
Waist circumference in cm	77.96	31.62	107	92	358.000	-7.215	<0.001
Serum HDL in mg/dl	45.55	82.16	42	48	678.000	-5.680	<0.001
Serum TG in mg/dl	83.62	27.00	215	144	98.000	-8.766	<0.001

In this table no 2, significance of difference between the study parameters in case and control group is analysed. Significant difference is found between case and control group in these study parameters. serum hs-CRP, serum gamma interferon, Serum TG, Serum HDL, plasma FBG, HOMAIR, waist circumference.

Table 3: Non parametric correlation analysis to the significance of strength between different parameters of the case group:

			HOMA-IR	FBS (mg/dl)	hs-CRP (mg/l)	Gamma IFN (pg/ml)	Waist circumference in cm
Spearman's rho	HOMA-IR	Correlation Coefficient	1.000	.361**	.530**	.334**	.168
		Sig. (2-tailed)	.	.002	<0.001	.004	.162
		N	71	71	71	71	71
	FBS (mg/dl)	Correlation Coefficient	.361**	1.000	.085	-.053	.048
		Sig. (2-tailed)	.002	.	.479	.662	.694
		N	71	71	71	71	71
	hsCRP (mg/l)	Correlation Coefficient	.530**	.085	1.000	.257*	.127
		Sig. (2-tailed)	<0.001	.479	.	.030	.291
		N	71	71	71	71	71
	Gamma IFN (Pg/ml)	Correlation Coefficient	.334**	-.053	.257*	1.000	.150
		Sig. (2-tailed)	.004	.662	.030	.	.211
		N	71	71	71	71	71
	WC (cm)	Correlation Coefficient	.168	.048	.127	.150	1.000
		Sig. (2-tailed)	.162	.694	.291	.211	.
		N	71	71	71	71	71

Results of the correlation study in Table 3 show that the IFN gamma is found to be positively correlated to the hs-CRP level. Correlation coefficient is 0.257 between IFN gamma and hs-CRP among case group with a p value of 0.030 indicating a close relationship between these two inflammatory markers. There is significant positive correlation of HOMA-IR with hs-CRP (correlation coefficient 0.530, p <0.001) and with gamma interferon (correlation coefficient 0.334, p =0.004).

DISCUSSION

Many studies have provided enough evidences to suggest that MS is a clustering of risk factors like obesity, DM, IR, hypertension, dyslipidemia etc. There are so many proinflammatory markers which show positive correlation with MS and atherosclerosis as well as CAD, CVA. Proinflammatory markers like hs-CRP and cytokines like IFN-gamma are associated with MS [6,23-25].These inflammatory markers are also responsible in causation of CAD, CVD by promoting atherosclerosis.

The values from the Table 1 C and 2 show significantly higher values of HOMA-IR and waist circumference in the case group that strongly validate the diagnosis of MS in our case group. These tables show TG level is significantly higher and HDL level is significantly lower in case group in comparison to control groups, which also strongly validate the diagnosis of MS in our case group [26]. Table 1A and 1B show that there is no significant difference in sex and age parameters between case and control groups, so it can be said that this study is age and sex matched. In the present study, hs-CRP level has been found to be significantly elevated in MS patients (Table 1C and 2). 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. Role of CRP in association with atherosclerosis is well documented in variety of race and age group [27,28]. Hs-CRP is far more specific than CRP in detecting vascular inflammation. Hs-CRP is also more sensitive than CRP for detecting low levels of chronic inflammation associated with heart disease [29,30-31].IR is associated with increased IFN gamma production in obese people and may therefore be an early step in the development of atherosclerosis in this population [32]. IL-18 induces IFN gamma production and IL-18 is increased in MS, so it can be said and it is evident that gamma interferon level is increased in patients of MS [33].

In our study hs-CRP is positively correlated with gamma interferon(r =0.257) (Table no 3) and a significant positive correlation is found between HOMA-IR and gamma interferon (r =0.334 and p value 0.004 in 2 tailed study) (Table no 3), thereby indicating role of these two proinflammatory markers in causation of IR. Differences in mean rank and median values of the parameters were found between the case and control population, which suggest a significant difference in the distribution of study parameters between the case and control population (Table 1C and 2). The result was validated by the Mann-Whitney test for determination of the significance of difference. Mann-Whitney test results show that FBS, hs-CRP, gamma interferon, waist circumference, and HOMA-IR levels are significantly higher in the case group. There is significant difference in TG and HDL level between case and control. These findings strongly suggested that the selected proinflammatory markers in our study population were significantly higher in the case group.

CONCLUSION

The present study was proposed to validate the result in Indian scenario with an object to explore the role of inflammatory panel markers like hs-CRP and cytokines like IFN-gamma in proved cases of MS of Indian origin. These results have important implications on the complications of MS. From the above results it can be concluded that, in patients of MS, hs-CRP and IFN-gamma level are increased and they have important role for causation of CAD, CVA.

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