“Relation of metabolic syndrome with inflammatory markers in non-diabetic first degree relatives of type 2 diabetes patients”

ABSTRACT

BACKGROUND

Vulnerability to type 2 diabetes is inherited, but its overt appearance is affected by medical, social and behavioral factors. It develops gradually over decades, with a long prediabetic interval of mild, moderate or intermittent hyperglycemia. The complexity of the natural history of type 2 diabetes and the high variability in its clinical presentation often results in about a decade’s delay before the diagnosis is made. A strong familial aggregation is observed among Asian Indians with high prevalence among the first degree relatives (FDRs). Although genetic predisposition largely governs the occurrence of type 2 diabetes, the exact molecular basis of defective insulin secretion and reduced tissue response to insulin remains elusive. In about 20% of cases, diabetic complications are seen at the time of initial diagnosis, indicating that the pathology begins much earlier. The exact time is difficult to ascertain and so far, alterations in the plasma glucose concentrations are the earliest markers.

The hypothesis that type 2 diabetes could be a disease of the innate immune system is another step towards understanding the pathophysiology of the disease.

Deficient insulin secretion and action has also been demonstrated in non-diabetic relatives of patients with type 2 diabetes. Inflammatory markers like the white blood cell count, CRP, Interleukins, fibrinogen and sialic acid have shown to predict the development of type 2 diabetes.

The need of the hour is to diagnose diabetes at the earliest, long before it is clinically evident and to arrest or delay its progress to complications. The role of inflammation in the etiopathogenesis of type 2 diabetes and the associated features of the metabolic syndrome
point to new approaches in the prediction and management of type 2 diabetes and also in the development of interventional therapies.

This study was designed as a cross-sectional case-control study to know whether non-diabetic first degree relatives (siblings and offspring) of patients with T2D manifest an inflammatory milieu as compared to subjects without a family history (FH) and to study the relation of Metabolic Syndrome (MetS) with inflammatory markers among the subjects with or without FH of diabetes.

**Key words**: Type2 Diabetes, First degree relatives, Inflammatory markers, Metabolic syndrome

**MATERIALS & METHODS**

In this cross-sectional case-control study, apparently healthy, as yet undiagnosed first degree relatives of type 2 diabetic patients of either sex in the age group of 20-60 years served as the test group (FH+) and were compared with those without a family history (FH−). The two groups FH+ and FH− were further dichotomized based on the age. Hence, the younger subjects aged 20-39 years with a positive family history were termed FHY+ and the older subjects aged 40-60 years with a positive family history were termed FHE+. Similarly, the age matched subjects with a negative FH were termed FHY− and FHE− respectively.

Pregnant women, chronic alcoholics, patients with chronic inflammatory diseases and subjects with history of active infection in the past 3 months were not enrolled in the study.

A total of 301 subjects participated in the study. The following parameters were collected/estimated/calculated.

**Demographic details**: age, height (Ht), weight (Wt), Waist Circumference (WC), Hip Circumference (HC), Blood Pressure (BP), Waist-Hip Ratio (WHR) and Body Mass Index (BMI)

**Biochemical investigations**: Fasting Plasma Glucose (FPG), 2 hr postload glucose (2 hr PG), Glycated Haemoglobin (HbA1c), Fasting Insulin (FIn), Homeostasis Model Assessment – Insulin Resistance (HOMA-IR), Fasting Lipid Profile (FLP), Total Leucocyte count (TLC), Erythrocyte Sedimentation Rate (ESR), Highly Sensitive C-reactive Protein (hsCRP), Ceruloplasmin, Haptoglobin and Fibrinogen. The NCEP-ATP III criteria & SAM NCEP-ATP III criteria were used to study MetS. In 47 FH+ and 48 FH− subjects selected randomly from
the above groups, additionally morning Serum Cortisol and Interleukin-6 (IL-6) tests were performed.

All estimations were carried out by standard procedures using Roche diagnostic kits adopted for auto analysers. ESR was estimated by Westergren’s method on VESmatic 20, TLC by flow cytometry on Sysmex XT-1800 and fibrinogen by the Clauss clotting time method.

Statistics: The results of the study were analysed using statistical package for social science (SPSS) version 11.5 (Chicago IL). Results were presented as mean ± SD. Means of the normally distributed variables were compared using student’s unpaired ‘t’ test. The values of hsCRP levels were skewed across all the groups. Hence, the nonparametric test Mann Whitney ‘u’ test was used for hsCRP and the results expressed are ‘z’ score. A ‘p’ value <0.05 was considered significant. All categorical data were analysed by chi square test ($\chi^2$ test). Certain demographic data, categorical data and frequencies are expressed as proportions (%). Pearsons correlational analysis was done to estimate univariate association of the various parameters. ‘r’ value >0.2 was considered significant and ‘r’ value >0.3 was defined as a strong association. Multiple regression analyses were performed to examine risk factors for MetS separately in both the criteria and also to check for associations of the anthropometric and metabolic variables with inflammation markers. The significance level was set at 5%. The predictive efficacy of each MetS parameter was assessed by calculating the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) using the cut points specified for the MetS variables in the two criteria.

RESULTS & DISCUSSION

Comparisons of FH$^+$ and FH$^-$ groups yielded the following results:

1. IL-6 and cortisol were found elevated in FH$^+$. Mean values of TLC, ESR, hsCRP, ceruloplasmin, haptoglobin and fibrinogen did not differ in the two groups. Peripheral circulating acute phase proteins may not be suitable predictors of diabetes development in non-diabetic FDRs. The use of IL-6 needs to be confirmed in larger sample size.

2. FH$^+$ subjects were heavier, had significantly more abdominal fats as compared to FH$^-$ subjects.

3. They had elevated HbA$_{1c}$ levels indicating higher mean plasma glucose with greater degree of peripheral IR and compensatory hyperinsulinemia. Higher LDL-L and greater TC/HDL ratio point to proatherogenic lipid perturbations in them.
4. Age, BMI, WC, PPG, HbA1c, Insulin and Homa-IR correlated with inflammation markers in FH+. Among FH− subjects, along with the above, SBP, DBP, Tg and HDL also correlated with inflammatory markers and the strength of association was stronger in the FH− group.

5. In the sub-group with additional cortisol and IL-6 estimations, IL-6 correlated with BMI, WC and PPG in the FH+ and with BMI, WC, PPG, Fln and Homa-IR in the FH− groups. Again the strength of association was stronger in the FH− group.

6. BMI and WC strongly affected changes in PPG, Fln, Insulin and Homa-IR among the FH+ subjects. SBP and DBP also correlated with Fln and Homa-IR.

7. Strong positive associations of FPG, PPG, HbA1c, SBP, DBP, Tg and negative association of HDL-C was seen with Fln and IR in the FH+ group as compared to FH− group, reiterating the common soil hypothesis that even mild alterations in the glycemic, lipid and blood pressure status share an underlying insulin resistance pathology in the nondiabetic subjects and the mechanism is pronounced in those with a family history of diabetes.

Gain in weight and central fat deposition provokes insulin resistance and hyperinsulinemia which contributes to deleterious lipid disturbances. Both these bring about disharmony of the glucose homeostasis and blood pressure control. The above derangements are probably the earliest noticeable changes in subjects at risk of developing diabetes i.e., FDRs of T2D patients. These derangements activate the inflammatory process causing elevations of IL-6. But, counter regulatory mechanisms like hypercortisolism and hyperinsulinemia can still control the inflammation and inhibit the peripheral acute phase response. The chick and egg theory exists with inflammation markers and hyperglycemia. It has been proposed by a few that hyperglycemia invokes/exacerbates an inflammatory response, while the converse is advocated by some. The results of the present study conducted in nondiabetic FDRs of T2DM patients support the theory that inflammation (if any) is a consequence of excess adiposity, abnormal glucose homeostasis and lipid aberrations.

Cumulative evidence from the comparisons of FHY+, FHY−, FHE+ and FHE− groups suggests

1. The FH+ subjects – both FHY+ and FHE+ have higher visceral fat as compared to FHY− and FHE− respectively.

2. Although not statistically significant, with the sample size chosen here, adverse alterations in most of the studied parameters were observed in the FH+ group.
3. The FHY$^+$ group is a particularly susceptible one and the findings imply a more profound and protracted effect on the course of diabetes as and when they convert.

4. In due course, among the plasma glucose, FPG is the first to alter associated with hyperinsulinemia/insulin resistance.

5. The extent to which WC, FPG, HbA$_{1c}$ and IR were elevated in the FHE$^+$ group was not sufficient to provoke a strong inflammatory response in them although these factors are implied in many studies, thus necessitating a higher degree of impairment of the above parameters to provoke an inflammatory response.

6. Inflammatory markers may not be suitable as predictors of diabetes development in both younger and older individuals with a FH of diabetes. Their role, however, in older individuals without a FH needs to be ascertained with a larger sampling.

Drastic differences were not found in the anthropometric and the metabolic variables between the younger and the older subjects with a FH of diabetes. Similar comparisons in the subjects without a FH of diabetes showed significant age related adverse changes in the older individuals. The younger group with FH already have a deranged anthropo-metabolic profile because of which they do not differ significantly from their older counterparts. This indicates a possibility of the FHY$^+$ subjects developing diabetes early thereby having a prolonged and protracted course of the disease with impending complications. The findings impress the need to particularly monitor this group.

From the results in MetS subjects it can be summarized that, the subjects with FH of diabetes are more prone to MetS compared to those without a FH. But once MetS develops, the clinico-biochemical profile does not vary between the two sets of individuals. However, those with a FH have greater visceral adiposity. Inflammatory markers are raised in MetS cases classified by both ATP III and SAM-NCEP criteria. Application of the SAM-NCEP criteria to nondiabetic, not so obese subjects improves the sensitivity of identifying the MetS cases. WC appears to be the single most MetS candidate constituent to be associated with inflammation. Hence, this alone may suffice in the determination of CVD risk said to be conferred by MetS.

**CONCLUSION**

To conclude, among non-diabetic first degree relatives of type 2 diabetes patients, inflammatory proteins are not elevated and thus can not be used as markers of diabetes risk in
the clinical settings. Although significant elevations of IL-6 were observed, it is not possible to favour a unifying hypothesis in this regard because of the representative sample size. The traditional risk factors, BMI, WC, atherogenic lipids and HbA1c routinely measured are better suited as risk predictors. Metabolic syndrome is higher in them and is also associated with inflammation. Waist circumference is the MetS constituent which strongly correlates with inflammation. Since it is said that the FDRs are at 50-70% risk of developing diabetes, it is worthwhile to monitor them especially the younger FDRs who are the most vulnerable group.