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Correlation of Plasma C-reactive Protein Levels to Sialic Acid and Lipid Concentrations in the Normal Population

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Aim of this study was to investigate the relationship between sialic acid component and C-reactive protein and lipids in the plasma of 80 healthy subjects. Levels of sialic acid, C-Reactive Protein (CRP) and plasma lipids were measured in a normal population consisting of 80 subjects. The possible correlation between sialic acid and CRP was also studied. The total sialic acid concentration was 2.61±0.61 mM L^{-1}, CRP (2.52±2.32 mmole L^{-1}), total cholesterol (5.50±1.28 mM L^{-1}), triglycerides (1.31±0.87 mM L^{-1}), low density lipoprotein (3.51±1.11 mM L^{-1}) and high density lipoprotein (1.40±0.37 mM L^{-1}). There was a positive correlation between sialic acid and CRP ($r = 0.283$, $p<0.05$). However, there was no correlation between CRP and the plasma lipids.

Key words: Sialic acid, C-reactive protein, type 2 diabetes, lipids

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INTRODUCTION

The cause of Type 2 diabetes mellitus (non-insulin dependent) which affects millions of people throughout the world is not known. One of the foremost challenges is not only to account for hyperglycaemia, but also for the other biochemical abnormalities, which together with glucose tolerance, has been defined as metabolic syndrome X (Wajchenberg *et al*., 1994; Reaven, 1988). Although it is well established that insulin resistance and impaired insulin secretion are central to the pathogenesis of type 2 diabetes, it has been unclear how these abnormalities are related to the other biochemical features common in type 2 diabetes including central obesity, hypertension, accelerated atherosclerosis and dyslipidemia. However, inflammation has been implicated as part of the insulin resistance syndrome (Festa *et al*., 2000; Frohlich *et al*., 2000) and plays an important role in the onset and development and evolution of atherosclerotic lesions (Maseri, 1997).

There is increasing evidence that an ongoing cytokine induced acute phase response sometimes known as low grade inflammation is closely involved in the pathogenesis of type 2 diabetes and associated complications such as dyslipidemia and atherosclerosis (Pickup, 2004). Elevated circulating inflammatory markers such as C-Reactive Protein (CRP) and interleukin-6 predict the development of type 2 diabetes (Schmidt *et al*., 1999). It is also known that CRP induces adhesion molecule expression in human endothelial cells in the presence of serum. These findings support the hypothesis that CRP may play a direct role in promoting the inflammatory component of atherosclerosis (Koenig *et al*.,1999; Lagrand *et al*., 1999). Previous reports suggest a positive association between components of the Insulin Resistance Syndrome (IRS) and the acute phase proteins, including CRP (Pickup, 2004). Lindberg *et al*. (1991) have shown that an elevated total serum sialic acid concentration is a risk factor for cardiovascular mortality in the general population. Nayak and Roberts (2006) have shown that serum sialic acid may be used as an inflammatory marker and possible indicator of complications among the Caribbean Type 2 diabetics. Since CRP is part of the acute phase response and sialic acid is present at the end of the carbohydrate moiety of the acute phase proteins (Lindberg *et al*., 1991), the objective of this study was to investigate the relationship between the sialic acid component and C-reactive protein in the plasma of 80 healthy subjects.

MATERIALS AND METHODS

Subjects: Eighty healthy subjects were chosen from Klang Valley, Kuala Lumpur, through the distribution of questionnaires. Subjects Included were those without a family history of diabetes, hypertension, coronary artery disease and a body mass index of less than or equal to 30 kg m^{-2} without other cardiovascular risk factors. Equal number of males and females were chosen.

Methods: Fasting blood was collected in bottles containing disodium Ethylene Diamine Tetraacetate (EDTA) and the plasma was separated immediately by centrifugation at 3000 rpm for 15 min at 4°C. Total Cholesterol (TCH), triglycerides and High Density Lipoprotein (HDL) levels were determined using the individual biochemical kits supplied with Dimension^R clinical chemistry system (Dode Behring, France) and Low Density Lipoprotein Levels (LDL) were determined by the Friedewald equation (Friedewald *et al*., 1972). Sialic acid was determined by the modification of the periodate resorcinol method as described by Jourdian *et al.* (1971) and CRP determined using Active CRP ELISA kit (Diagnostic Systems Laboratories, Webster) according to the manufacturer's instructions.

VCAM-1 was measured using hsVCAM-1 ELISA kit (Boehringer Mannheim GmbH, Germany).

Statistical analysis: Data were expressed as mean±standard deviation. Bivariate Correlation (Pearson correlation coefficient) was used to study the correlation. Two tailed p-value of less than 0.05 was considered significant.

RESULTS

All subjects in this study were of comparable age and did not differ significantly in their body mass index. The mean concentrations of CRP, total sialic acid, TCH, triglycerides, HDL and LDL in the samples were

Table 1: Mean±SD of CRP, total sialic acid and plasma lipid concentrations

CRP	Sialic acid	TCH	Triglycerides	LDL	HDL
(μg ml^{-1})	----------	----------	mM L^{-1}	----------	----------
2.52±2.32	2.61±0.61	5.50±1.28	1.3±0.87	3.51±1.11	1.40±0.37

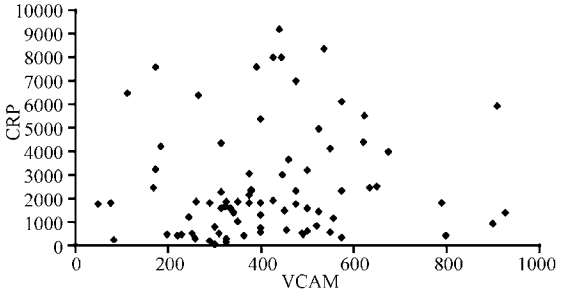

Fig. 1: Scatter plot of CRP vs VCAM-1

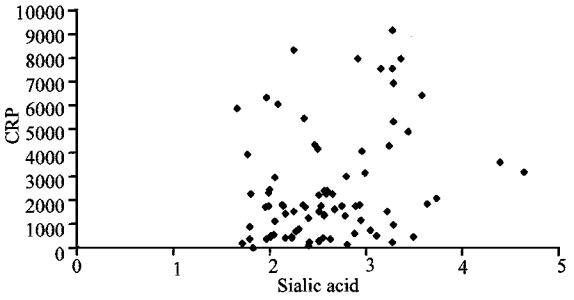

Fig. 2: Scatter plot of CRP vs Sialic Acid

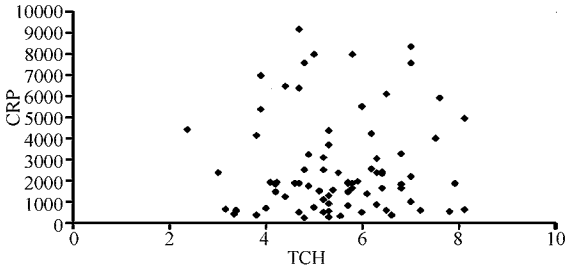

Fig. 3: Scatter plot of CRP vs TCH

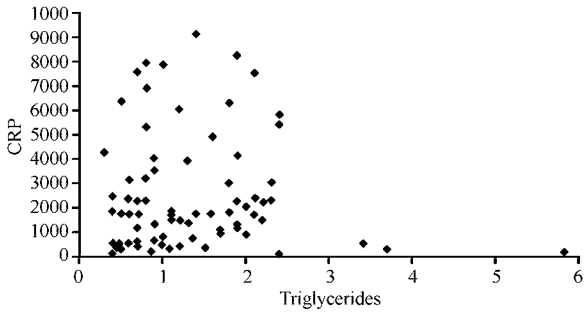

Fig. 4: Scatter plot of CRP vs Triglycerides

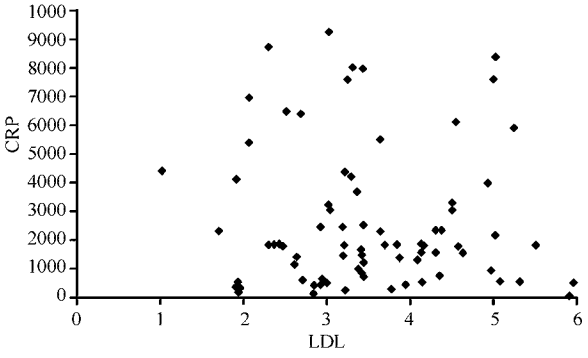

Fig. 5: Scatter plot of CRP vs LDL

determined (Table 1). Figure 1-6 show scatter plots of CRP levels versus VCAM-1, versus sialic acid and lipid levels. Figure 1 shows a scatter plot of the correlation between

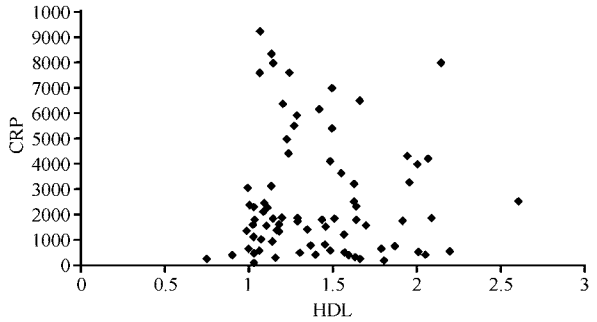

Fig. 6: Scatter plot of CRP vs HDL

Table 2: Pearson correlation between CRP and the parameters VCAM1, sialic acid and plasma lipid concentrations

Parameter	VCAM1	Sialic acid	TCH	TRIG	LDL	HDL
CRP	0.095	0.283*	0.018	-0.036	0.035	0.007

*Significant at $p<0.05$

CRP and VCAM-1. There was a significant positive correlation between sialic acid and CRP ($r = 0.283$, $p<0.05$) among the 80 healthy subjects recruited (Table 2). Figure 1 shows a weak correlation between CRP and VCAM-1 ($r = 0.095$). Figure 3-6 shows a lack of correlation between CRP and TCH, triglycerides, LDL and HDL respectively.

DISCUSSION

Type 2 diabetes mellitus and atherosclerotic cardiovascular disease share many antecedent factors that frequently coexist, which has given rise to the concept of common soil (Jarrett and Shiply, 1988; Schmidt *et al*., 1996; Stern, 1995). This cluster of risk factors, such as uric acid and dyslipidaemia, are strongly related to fasting insulin concentrations and central obesity (Schmidt, 1996) and are also associated with raised concentration of inflammatory markers in people with and without diabetes (Yudkin, 1997; Pickup *et al*., 1997).

Inflammatory processes play a part in the cause of atherosclerotic cardiovascular disease (Ross, 1999). Concentrations of acute-phase response markers and mediators of inflammation such as tumor necrosis factor-alpha-1 (TNFα1) and interleukin-6 are raised in people with type 2 diabetes (Pickup *et al*., 1997; Nilsson *et al*., 1998). It has also been shown that some of the inflammatory markers such as CRP and sialic acid are increased in type 2 diabetes.

CRP, the classic acute-phase reactant, is an extremely sensitive systemic marker of inflammation (Chambers *et al*., 2001). It is a part of the immune response to injury and infection and it was shown to be an

independent predictor of risk for the development of diabetes (Freeman, 2002). Sialic acid, a family of acetylated derivatives of neuraminic acid, is widely distributed in mammals. It usually occurs as a terminal component at the non-reducing end of carbohydrate chain of glycoproteins and glycolipids (Ng and Dain, 1976).

In present study, there was a good correlation between the C-reactive protein and sialic acid. Although we believe the link between cytokines and the acute phase response to be the most likely explanation for the association, some association may reflect other pathways linking acute phase reactants to alteration in insulin sensitivity or secretion. For instance removal of cell membrane sialic acid (perhaps through processes that shed sialic acid into the circulation, thereby raising its concentration) has been shown experimentally to induce insulin resistance (Salhanick and Amatruda, 1988).

Most of the acute phase proteins are glycoprotein with notable amount of sialic acid. In a Swedish population, orosomucoid, haptoglobin and α1-antitrypsin concentrations together explained 70% of the variability of total serum sialic acid concentration (Lindberg *et al*., 1993). Some studies have shown that the sialic acid concentrations are elevated in diabetics of both type 1 and type 2 with and without complications (Nayak and Roberts, 2006) while others have reported no such correlation (Abdella *et al*., 2000; Crook *et al*., 2002). Studies have also found that the presence or absence of this trend may be related to ethnicity (Lindberg *et al*., 1997). These findings have led to the suggestion that raised concentrations of sialic acid from glycoproteins and the resultant acute phase response may underlie much of the metabolic clustering, including glucose intolerance (Pickup and Crook, 1998).

A better understanding of cytokine actions and interactions with other factors in the pathogenesis of type 2 diabetes may lead to improved understanding of its causes and open new approaches for its prevention.

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