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RESEARCH ARTICLE

ASSOCIATION OF GENETIC MARKERS OF CARDIOVASCULAR DISEASE WITH TYPE 2 DIABETES

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ABSTRACT

Introduction: One of the most frequently occurring macro vascular complications is cardiovascular disease (CVD). It is a leading cause of morbidity and mortality in many countries worldwide and is estimated that it will be the single largest cause of disease burden globally by the year 2020. Diabetes mellitus (DM) is found to be the major risk factor for cardiovascular disease (CVD). As compared with individuals without diabetes, CVD is 2–4 times more common in people with diabetes and is associated with a higher mortality.

Objective: The main objective of the present study is to observe the association of CVD with type 2 diabetes and genetic markers such as plasma proteins namely, Haptoglobin [HP], Group specific component [GC], Transferrin [TF], Albumin [ALB] and Caeruloplasmin [CP] systems.

Materials and Methods: In the present study, fifty cases presenting cardio vascular disease with type 2 diabetes mellitus and fifty cases of age and sex matched healthy controls were included. Plasma samples were typed using PAGE electrophoresis. The statistical significance of differences between patients and controls were tested. Analysis of the data was carried out using Epi Info 5 software. Relative risk was calculated by the random-effects method.

Results: The study was observed that in the case of CVD with diabetes type 2 patients were showing significant association for HP (χ^2:6.2864; d.f.= 2;0.05>p>0.02) and GC (χ^2:10.0150; d.f.= 2; 0.01> p> 0.001) systems. Risk estimates showed a significant association of HP 2-2 and GC 2-1 phenotypes with CVD-type 2 diabetes mellitus individuals (RR = 2.23). The result shows an increased risk of 100% and more, indicating that individuals with these phenotypes were two times more likely to get the disease when compared with the other phenotypes of the HP and GC systems.

INTRODUCTION

A number of genetic polymorphisms exist in human beings, which manifest variable susceptibilities towards pathogenesis and etiology of a particular disease. Some genetic markers might be serving some hidden important biological functions for understanding biological significance of polymorphisms in man. Special attention is being diverted towards the relationship between the genetic markers and human diseases. A biochemical marker will influence disease susceptibility which implies that some product related to gene determining biochemical trait or possibly the product of some closely linked genes take part in the complex mechanism influencing diseases. Several diseases have been studied for their association with various genetic markers (Mourant *et al*., 1978). Diabetes Mellitus (DM) is a worldwide problem and the most common endocrine disorder. Its prevalence is increasing in the present scenario of a sedentary lifestyle in the general population. Worldwide, prevalence of type 2 diabetes mellitus has been estimated to rise from 150 million to 225 million by the end of 2010 and to as many as 300 million by 2025 (Zimmet 2003; Wild *et al*., 2004). Similarly, in India this increase is estimated to be 58%, from 51 million people in 2010 to 87 million in 2030 (Snehalatha and Ramachnadaran, 2009). The hyperglycemia resulting from impaired secretion or function of insulin can lead to metabolic, vascular, neurological and immunological abnormalities which can be largely divided into macro vascular and micro vascular complications. The macro vascular complications include cerebrovascular disease, coronary heart disease, and peripheral vascular disease. The micro vascular complications include diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy. One of the most frequently occurring macro vascular complications is Cardiovascular Disease (CVD). It is a leading cause of morbidity and mortality in many countries worldwide and is estimated that it will be the single largest cause of disease burden globally by the year 2020. Epidemiologists in India and international agencies, such as the World Health Organization, have been sounding an alarm on the rapidly rising burdens of CVD for the past fifteen years (Reddy, 1993, Reddy *et al*., 2005, WHO, 2005). Analysis of the data-subset related to five South Asian countries, including a large fraction from India, showed clearly that the nine risk factors, which were identified as being strongly associated with CVD across fifty two countries, were also operative in South Asians. It also showed that differences in these risk factors explain why Indians experience their first heart attack at an earlier age than other populations (Joshi *et al.,* 2007). The

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recognition that behavioral risk factors like tobacco, physical inactivity, and unhealthy diets can no longer be ignored and the realization that biological risk factors like blood pressure, diabetes and dyslipidemia need to be appropriately and adequately controlled have at last opened the strategic pathways for CVD prevention and control in India. Out of these, Diabetes Mellitus (DM) is found to be the major risk factor for Cardiovascular Disease (CVD), including Coronary Artery Disease (CAD), stroke and peripheral arterial disease. As compared with individuals without DM, CVD is 2–4 times more common in people with diabetes and is associated with a higher mortality (Kannel and Gee, 1979; Stamler *et al*., 1993). Recent advances in the field of molecular biology have led to a better understanding of the pathological mechanisms of cardiovascular disease (Cambien *et al*., 1999; Ellsworth *et al*., 1999). The impact of these findings will shape the future treatment modalities for cardiovascular disorders. Biochemical genetic markers are of considerable importance in disease association studies. The existence of genetically determined polymorphisms of plasma proteins has led to more number of investigations into the possible correlations between these genetic markers and human diseases. We report here the polymorphism data of five plasma proteins, Haptoglobin (HP), Caeruloplasmin (CP), Group Specific Component (GC), Transferrin (TF) and Albumin (ALB). The main objective of the present study is to determine whether these genetic markers are predictors of CVD in patients with diabetes.

MATERIALS AND METHODS

Blood samples from a total of 50 CVD patients with diabetes and 50 healthy, normal individuals of both sexes were collected. The samples were collected from local hospitals of Visakhapatnam city, North Coastal Andhra Pradesh, South India. In this study five genetic markers such as plasma proteins which include Albumin (ALB), Haptoglobin (HP), Caeruloplasmin (CP), Group Specific Component (GC) and Transferrin (TF) were studied. 3ml of intravenous blood samples were collected in sterile test tubes containing ACD solution as an anticoagulant. The samples were brought to the laboratory in a thermos flask containing ice, within few hours of sample collection. The Plasma was separated. Fresh and clear hemolysates were prepared according to standard procedures and stored until further use. The plasma protein markers - Group Specific Component (GC), Transferrin (TF) and Albumin (ALB) were typed by acrylamide gel electrophoresis (Kitchin and Bearn, 1966) and Haptoglobin (HP) and Caeruloplasmin (CP) as described by Clark (1964).

The allele frequencies were estimated by maximum likelihood method (Balakrishnan, 1988) and statistical heterogeneity was tested using the standard χ^2 test (Taylor and Prior, 1938). Analysis of the data was carried out using Epi Info 5 software. Odds ratios and 95% confidence interval (95% CI) were calculated to assess the strength of the relationship between the biochemical markers and CVD with diabetes. Pooled odds ratios and relative risk were calculated by the random-effects method of DerSimonian and Laird (DerSimonian and Laird, 1986). Estimates from the random effects model incorporate the variability among studies and represent a more conservative approach. For odds ratio, confidence interval was calculated. Increased risk was calculated using the formula: Increased Risk = (Relative Risk – 1.00) x 100. The significance level was 5%.

RESULTS

Distribution of phenotypes and allele frequencies of genetic markers are shown in Table -1and 2 respectively. Considering haptoglobins, the study population showed a significant difference in their distribution when observed between patients and controls. There was a predominant occurrence of Haptoglobin 2 phenotype in patients when compared to controls. The heterogeneity test is statistically significant (χ^2: 6.2864; d.f. = 2; 0.05> p> 0.02). The distribution of the haptoglobin phenotypes in the case-control samples was not in consistent with the Hardy-Weinberg expectation (χ^2: 4.4610; d.f. = 1; 0.05> p> 0.02 and χ^2: 7.5616; d.f. = 1; 0.01>p>0.001).

Table 1. Plasma protein phenotypes in CVD-DM and controls

System	Phenotype	CVD-DM (Patients)		Controls	
		Obs.	Exp.	Obs.	Exp.
HP	1-1	00	02.64	02	06.48
	2-1	23	17.71	32	23.04
	2-2	27	29.65	16	20.48
	Total	**50**	**50.00**	**50**	**50.00**
		χ^2= 4.4610 (0.05>p>0.02)		χ^2= 7.5616 (0.01>p>0.001)	
GC	1-1	31	30.42	45	45.12
	2-1	16	17.16	05	04.75
	2-2	03	02.42	00	00.13
	Total	**50**	**50.00**	**50**	**50.00**
		χ^2= 0.2284 (0.70>p>0.50)		χ^2= 0.1388 (0.80>p>0.70)	
	C	50	50.00	49	49.00
	CD	-	-	01	01.00
TF	D	-	-	00	00.00
	Total	**50**	**50.00**	**50**	**50.00**
				χ^2= 0.8050 (0.50>p>0.30)	
CP	B	50	50.00	50	50.00
	TO **TAL**	50	50.00	50	50.00
ALB	N	50	50.00	50	50.00
	TOTAL	**50**	**50.00**	**50**	**50.00**

Table 2. **Allele frequencies in CVD-DM patients and controls**

System (Allele)		CVD-DM	Controls	Intergroup Heterogeneity	d.f
HP	1	0.2300 ± 0.0420	0.3600 ±0.0480		
2		0.7700 ± 0.0420	0.6400± 0.0480	6.2864	2
GC	1	0.7800 ± 0.0414	0.9500 ± 0.0217		
2		0.2200 ± 0.0414	0.0500 ± 0.0217	11.3406	2
TF	C	0.9900 ± 0.0099	1.0000 ± 0.0000		
CD		0.0100 ± 0.0099	-	1.0100	2
D		-	-		
CP	B	1.0000 ± 0.0000	1.0000 ± 0.0000		
ALB	N	1.0000 ± 0.0000	1.0000 ± 0.0000		

In the GC system, there was a significant difference observed between patients and controls. There was a predominant occurrence of GC 2-1 phenotype in patients when compared to controls, which had an increased frequency of 1-1 phenotype. A considerable decrease in the frequency of the GC*2 phenotype was observed in the patients, which could not be traced in the controls. The heterogeneity test is statistically significant (χ^2: 10.0150; d.f. = 2; 0.01> p> 0.001), indicating the presence of an association with GC system in CVD-DM individuals. No variation and no evident association was found with respect to other systems like CP, TF and ALB markers in the present study in both CVD-DM patients and controls. On the other hand, the caeruloplasmin (CP) and albumin (ALB) loci were monomorphic showing only the BB and N phenotypes

respectively. Test of association, odds ratio and relative risks of HP and GC phenotypes with the disease condition compared to the control group are presented in Tables 3 and 4 respectively.

An increased predisposition of homozygous HP 2-2 phenotype was observed in individuals with CVD ($\chi 2$ = 4.9400*). Homozygous 2-2 individuals were at increased risk of CVD, with an overall odds ratio of 2.49 (95% Confidence Interval: 1.03 - 6.11). Risk estimates show a significant association of HP 2-2 phenotype with CVD-DM individuals (RR = 1.69). The result shows an increased risk of 100% and more, indicating that individuals with 2-2 phenotype are nearly two times more likely to get the disease when compared with the other phenotypes of the haptoglobin. An increased predisposition of heterozygous GC 2-1 phenotype was observed in individuals with CVD ($\chi 2$ = 7.2900*). Heterozygous 2-1 individuals were at increased risk of CVD, with an overall odds ratio of 4.24 (95% Confidence Interval: 1.30 –16.05). Risk estimates show a significant association of GC 2-1 phenotype with CVD-DM individuals (RR = 3.20). The result shows an increased risk of 100% and more, indicating that individuals with 2-1 phenotype are three times more likely to get the disease when compared with the other phenotypes of the GC system.

Table 3. Test of Association, Relative Risk, Odds Ratio and 95% Confidence Interval Estimates of HP Phenotypes In CVD-DM and Control Group.

HP Phenotype combinations	Control (n)	CVD-DM					
		(n)	RR	OR	95% CI	χ^2 values	p-value
1-1 vs 2-1 + 2-2	2	0	0.00	0.00	0.00 –5.31	2.0400	0.1531
2-1 vs 1-1 + 2-2	32	23	0.00	0.48	0.20 –1.15	3.2700	0.0704
2-2 vs 1-1 + 2-1	16	27	1.69	2.49	1.03 –6.11	4.9400*	0.0262

Table 4. Test of Association, Relative Risk, Odds Ratio and 95% Confidence Interval Estimates of GC Phenotypes In CVD-DM and Control Group

GC Phenotype combinations	Control (n)	CVD-DM					
		(n)	RR	OR	95% CI	χ^2 values	p-value
1-1 vs 2-1 + 2-2	45	31	0.69	0.18	0.05 – 0.58	10.7500*	0.0010
2-1 vs 1-1 + 2-2	5	16	3.20	4.24	1.30 –16.05	7.2900*	0.0069
2-2 vs 1-1 + 2-1	0	3	0.00	0.00	0.00 – 0.00	3.0900	0.0786

DISCUSSION

Cardiovascular disease with Diabetes mellitus is a heterogeneous group of heart muscle disorders responsible for a great deal of morbidity and mortality. There are various biochemical and molecular applications for its identification and diagnosis. The present study is undergone through the biochemical approach to identify the association of various Plasma protein markers based on qualitative studies in Cardio vascular disease with Diabetes mellitus patients. Out of 5 genetic markers, only 2 markers- HP and GC were showing significant association with CVD-DM patients. The other genetic markers TF, CP and ALB were not showing any associations with cardiovascular disease patients having Diabetes mellitus. Haptoglobin, a hemoglobin-binding protein exists as two allelic variants designated 1 and 2, expressed by a genetic polymorphism as three major phenotypes: 1-1, 2-1 and 2-2. The HP class 2 allele present only in humans appears to have arisen from the HP 1 allele early in human evolution by a duplication of exons 3 and 4 of the HP 1 allele (Bowman and Kurosky, 1982; Langlois and Delanghe, 1966). A key difference between the alleles is that the protein product of the 1 allele is a more potent antioxidant compared with that produced by the 2 allele. It was demonstrated that haptoglobin phenotype is predictive of development of both micro vascular and macro vascular complications in DM (Levy *et al*., 2000; Nakhoul *et al*., 2000; Nakhoul *et al*., 2001). A Several longitudinal studies have provided evidence that the HP genotype is associated with diabetic cardiovascular complications. This case-control study demonstrates that haptoglobin phenotype is a significant predictor of CVD in individuals with DM. Individuals with the HP 2-2 phenotype had significantly two fold higher odds of CVD (OR=2.49, 95% CI=1.03–6.11) compared with individuals with the HP 2-1 or HP 1-1 phenotypes. Majority of studies till date reported association between HP and cardio vascular disease. The Strong Heart Study (SHS) among American Indians with CVD-DM, the HP 2-2 phenotype showed odds of almost fivefold (OR=4.96, 95% CI=1.85–3.33) compared to the HP 1-1 phenotype (Levy *et al*., 2002). Chapelle *et al* showed that following a myocardial infraction, the severity and extent of myocardial damage was much greater in patients with HP 2-2 than in those with HP 1-1 or HP 2-1 (Chapelle *et al*., 1982). Our findings are inconsistent with a recently reported prospective study from Belgium demonstrating increased cardiac mortality in patients with the HP 1-1 phenotype (DeBacquer *et al*., 2001). A major reason that the HP 2-2 genotype appears to be associated with cardiovascular disease in diabetes is thought to relate to the potential for increased oxidative damage caused by the HP (2-2) - HB complex, which contains redox-active iron and the steady-state plasma concentration of which is elevated compared to the complex formed by HP (1-1) – HB complex. Group specific component protein (vitamin D binding protein), which maps to chromosome 4 q.12, has been reported to be associated with non-insulin-dependent diabetes mellitus (NIDDM) and glucose metabolism in some populations (Mühlhauser *et al*., 1997; Malmberg, 1997). One possible mechanism would be that GC influences glucose metabolism by affecting the activity of vitamin D which might be correlated with glucose tolerance (Bijlstra *et al*., 1996).

Conclusion

Cardiovascular disease continues to disproportionately affect individuals with diabetes worldwide despite significant advances in prevention efforts and medical care for these patients. It has therefore become apparent that genetic predisposition plays a key role in the development of vascular complications in diabetes, and a functional polymorphism in the HP and GC genes has been identified as a potential determinant of vascular diabetes complication risk. This study

suggests that determination of haptoglobin phenotype may contribute to the algorithm used in CVD risk stratification, and in evaluation of new therapies to prevent CVD in the diabetic patient. It may be concluded that the marginal association of GC and HP alleles detected in the present study needs to be tested on a larger series.

REFERENCES

Balakrishnan, V., 1988. Hardy-Weinberg equilibrium and allele frequency estimation. In: KC Malhotra (Ed.): *Statistical Methods in Human Population Genetics*. Calcutta: *Indian Statistical Institute and Indian Society of Human Genetics*, pp. 39-93.

Bijlstra, P.J., Lutterman, J.A., Russel, F.G., Thien, T., Smits, P., 1996. Interaction of sulphonylurea derivates with vascular KATP channels in man. *Diabetologia,* 39: 1083-1090

Bowman, B.H., Kurosky, A., 1982, Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. *Adv Hum Genetics* 12:189–261.

Cambien, F., Poirier, O., Nicaud, V., Herrmann, S.M., Mallet, C., Ricard, S., Behague, I., Hallet, V., Blanc, H., Loukaci, V., Thillet, J., Evans, A., Ruidavets, J.B., Arveiler, D, Luc G., Tiret, L., 1999. Sequence diversity in 36 candidate genes for cardiovascular disorders. *Am J Hum Genet*, 1999, 65:183-191.

Chapelle, J.P., Albert, A., Smeets, J.P., Marechal, J.P., Heusghem C, Kulbertus H.E., 1982. Effect of the haptoglobin phenotype on the size of a myocardial infarct. *N Engl J Med,* 307:457–63.

Clark, J.T., 1964. Simplified "Disc" (Polyacrylamide) electrophoresis. *Ann NY Acad Sci,* 121: 428 - 436.

DeBacquer, D., DeBacker, G., Langlois, M., Delanghe, J., Kesteloot, H., Kornitzer, M., 2001. Haptoglobin polymorphism as a risk factor for coronary heart disease mortality. *Atherosclerosis*, 157:161–166.

DerSimonian, R., Laird, N., 1986. Meta-analysis in clinical trials. *Control Clin Trials,* 7: 77–88.

Ellsworth, D.L., Sholinsky, P., Jaquish, C., Fabsitz, R. R., Manolio, T.A., 1999. Coronary heart disease. At the interface of molecular genetics and preventive medicine. *Am J Prev Med*, 16: 122-133.

Joshi, P., Islam, S., Pais, P., *et al*. 2007. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *JAMA*, 297:286 –94.

Kannel, W., Mc Gee, D., 1979. Diabetes and cardiovascular disease: The Framingham Study. *JAMA,* 241:2035–8.

Kitchin, F.D., Bearn, A.G., 1966. The electrophoretic patterns of normal and variant phenotypes of the Group Specific Components (GC) in human serum. *Amer J Hum Genet,* 18: 201 -214.

Langlois, M.R., Delanghe, J.R., 1996. Biological and clinical significance of haptoglobin polymorphism in humans. *Clin Chem*, 42:1589–600.

Levy, A.P., Roguin, A., Hochberg, I., *et al*. 2000. Haptoglobin phenotype and vascular complications in diabetes. *N Eng J Med,* 343:969–70.

Malmberg, K., 1997. For the DIGAMI Study Group (1997) Prospective randomized study of intensive insulin treatment on long term survival after acute myocardial infarction in patients with diabetes mellitus. *Br Med J*, 314: 1512-1515.

Mühlhauser, I., Sawicki, P.T., Berger, M., 1997. Possible risk of sulphonylureas in the treatment of non-insulin-dependent diabetes mellitus and coronary artery disease (letter). *Diabetologia,* 40:1492-1493.

Mourant, A.E., Kopec, A.C., Domaniewska, S.K., 1978. Blood Groups and Diseases. London: Oxford University Press.

Nakhoul, F., Marsh, S., Hochberg, I., Leibu, R., Miller, B.P., Levy, A.P., 2000. Haptoglobin phenotype and diabetic retinopathy. *JAMA,* 284:1244–5.

Nakhoul, F., Zoabi, R., Kantor, Y., *et al*. 2001. Haptoglobin phenotype and diabetic nephropathy. *Diabetologia,*, 44:602–4.

Reddy, K.S., 1993. Cardiovascular disease in India. *World Health Stat Q*, 46:101–7.

Reddy, K.S., Shah, B., Varghese, C., Ramadoss, A., 2005. Responding to the challenge of chronic diseases in India. *Lancet,* 366:1744 –9.

Snehalatha., Ramachnadaran., 2009. Insight into the Mechanism of Primary Prevention of Type 2 Diabetes: Improvement in Insulin Sensitivity and Beta cell function. "Genetic and Epigenetic Basis of Complex Diseases "conference in Centre for Cellular and Molecular Biology.

Stamler, J., Vaccaro, O., Neaton, J.D., Wentworth, D., 1993. Diabetes, other risk factors, and 12-year cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care,* 16:434–44.

Taylor, G.L., Prior, A.M., 1938. Blood groups in England II distribution in the population. *Ann Eugen*, 8: 356 – 361.

Wild,H., Roglic, G., Green, A., Sicree, R., King, H., 2004. Global prevalence of diabetes, estimates for the year 2000 and projections for 2030. *Diabetes care*, 27: 1047-53.

World Health Organization. The World Health Report 2005. Preventing Chronic Diseases: A Vital Investment. Geneva: WHO, 2005.

Zimmet, P., 2003. The burden of type 2 diabetes: are we doing enough? *Diabetes Metab*, 29: 689-18.

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