© JLS 2013 J Life Science, 5(1): 1-4 (2013)
PRINT: ISSN 0975-1270 ONLINE: ISSN 2456-6306 DOI: 10.31901/24566306.2013/05.01.01

Association of Genetic Markers with Cervical Cancer

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KEYWORDS Cervical Cancer. Plasma Proteins. Polymorphism. Association

ABSTRACT Cervical cancer, a malignant neoplasm of the cervix uteri or cervical area is the second most common cause of cancer related deaths among women. This study is to determine whether the biochemical genetic markers are predictive of cervical cancer patients. Blood samples from 50 cervical cancer diseased patients and equal number of age and sex matched healthy individuals with no known history of any disease were taken as controls. In this study five genetic markers - which include Albumin (ALB), Haptoglobin (HP), Transferrin (TF), Caeruloplasmin (CP) and Group Specific Component (GC) were studied. The present study is an attempt to find association between cervical cancer and genetic markers like plasma proteins. For group specific component system, no significant differences were observed between patients and controls (χ^2: 5.7136; d.f= 2; $0.10 > p > 0.05$), and both the examined groups were in Hardy-Weinberg equilibrium indicating no association between cervical cancer and this protein marker. Regarding haptoglobins, a significant difference in their distribution was observed between patients and controls. This data shows an association of HP 2-2 in patients with cervical cancer. Thus HP system showed significant differences between patients and controls (χ^2: 7.6284; d.f= 2; $0.05 > p > 0.02$).

INTRODUCTION

Cancer is a complex disease involving multiple genetic and environmental risk factors. To clarify the contribution of genetic factors and decipher the relationship between genes, environment, and cancer, association studies are generating much genetic information. This has often taken the form of studying single nucleotide polymorphisms in different genes. Analyzing such data is challenging, and raises the issues of multiple comparisons and potential false-positive associations (Greenland and Rothman 1998).

Cervical cancer (CxCa) is the second leading cause of cancer-related deaths after breast cancer, for women between 20 and 39 years old (Landis et al. 1999). Infection by the human papillomavirus (HPV) is considered as the central risk factor for CxCa (Walboomers et al. 1999). However, it is unlikely to be the sole cause for developing cancer. Ongoing research investigates the role of specific genetic and environmental factors in determining HPV persistence and subsequent progression of the disease (Agorastos et al. 2005). In this context, genetic association studies constitute a significant scientific approach that may lead to a more comprehensive and holistic insight on the origin of complex diseases, such as CxCa (Hirschhorn et al. 2002). Genetic association studies aim to detect association between one or more genetic variants (for example, polymorphisms) and a trait, which might be some quantitative characteristic, a discrete attribute, or a disease (Cordell and Clayton 2005). A genetic variant is genotyped in a population for which phenotypic information is available (such as disease occurrence, or a range of different trait values). If a correlation is observed between genotype and phenotype, there is an association between the variant and the disease or trait (Hirschhorn and Daly 2005).

The main objective of the present study is to observe any association between various genetic markers like haptoglobin (HP), caeruloplasmin (CP), group specific component (GC), transferrin (TF) and albumin (ALB) in individuals who are affected with cervical cancer.

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MATERIAL AND METHODS

A total of 50 venous blood samples were collected from cervical cancer patients, diagnosed in Rangaraya Medical College, Kakinada, East Godavari District. Equal number of age and sex matched healthy individuals (50 females) with no known history of any disease were taken as controls.

In this study five genetic markers - serum proteins which include Albumin (ALB), Haptoglobin (HP), Transferrin (TF), Caeruloplasmin

Table 1: Distribution of plasma protein phenotypes in cervical cancer and controls

System	*Phenotype*	*Cervical cancer*		*Controls*	
		Observed	*Expected*	*Observed*	*Expected*
HP	1-1	0	0.41	2	2.64
	2-1	9	8.19	19	17.71
	2-2	41	41.40	29	29.65
	Total	*50*	*50.00*	*50*	*50.00*
		$\chi^2 = 0.4890$		$\chi^2 = 0.3341$	
		(0.80 > þ > 0.70)	(0.90 > þ > 0.80)		
GC	1-1	14	13.52	24	23.81
	2-1	24	24.96	21	21.39
	2-2	12	11.52	5	4.80
	Total	*50*	*50.00*	*50*	*50.00*
		$\chi^2 = 0.0739$		$\chi^2 = 0.0165$	
		(0.98 > þ > 0.95)	(1.0 > þ > 0.99)		
ALB	N	50	-	50	-
	Total	*50*	-	*50*	-
CP	B	50	-	50	-
	Total	*50*	-	*50*	-
TF	C	50	-	50	-
	Total	*50*	-	*50*	-

(CP) and Group Specific Component (GC) were studied. 5ml of venous blood was collected into sterilized test tubes containing EDTA as anti coagulant. The samples were brought to the laboratory in a thermos flask containing ice, within few hours of sample collection. The plasma was separated and haemolysates were prepared and stored at -20°C until use. The plasma protein markers - Group specific component (GC) and Albumin (ALB) and Transferrin (TF) were typed by acrylamide gel electrophoresis (Kitchin and Bearn 1966) and Haptoglobin (HP) and Caeruloplasmin (CP) as described by Clark (1964). The gene frequencies were estimated by using maximum likelihood methods of Balakrishnan (1988) and goodness of fit between the observed and expected phenotype frequencies were tested according to Taylor and Prior (1938). Analysis of data was carried out using Epi Info 5 software. In order to measure the extent of correlation, the odds ratio was applied. Pooled odds ratios and relative risk were calculated by the random-effects methods of Der Simonian and Laird (1986).

RESULTS AND DISCUSSION

Distribution of phenotypes and allele frequencies of genetic markers are shown in Table 1 and Table 2.

The allele frequencies of HP*1 and HP*2 in controls is found to be 0.2300 and 0.7700 and in cervical cancer patients is 0.0900 and 0.9100 respectively. The study population showed the predominant occurrence of HP 2-2 phenotype in cervical cancer patients. A decreased predisposition of heterozygous HP 2-1 phenotypic individuals were observed. Although the frequency of the HP*1 allele is found to be 0.0900, the Haptoglobin1-1 phenotype could not be traced in cervical cancer patients. The Chi-square test for homogeneity was found to be non-significant ($\chi^2 = 0.3341$; d.f = 1; 0.90 > p > 0.80) in controls with respect to cervical cancer patients ($\chi^2 = 0.4890$; d.f = 1; 0.80 > p > 0.70). The inter group heterogeneity was found to be ($\chi^2 = 7.6284$; d.f = 2; 0.05 > p > 0.02), a significant value when observed between cervical cancer patients and controls. Thus, due to association, a significant deviation

Table 2: Distribution of allele frequencies in cervical cancer and controls

System (allele)	*Cervical cancer*	*Control*	*Intergroup heterogeneity*	*d.f*
HP				
1	0.0900 ± 0.0286	0.2300 ± 0.0420	7.6284*	2
2	0.9100 ± 0.0286	0.7700 ± 0.0420		
GC				
1	0.5200 ± 0.0314	0.6971 ± 0.0318	5.7136	2
2	0.4800 ± 0.0314	0.3029 ± 0.0318		
CP				
B	1.0000 ± 0.0000	1.0000 ± 0.0000		
TF				
C	1.0000 ± 0.0000	1.0000 ± 0.0000		
ALB				
N	1.0000 ± 0.0000	1.0000 ± 0.0000		

from the Hardy-Weinberg equilibrium was found in the cervical cancer patients.

Haptoglobin is a hemoglobin-binding protein expressed by a genetic polymorphism as three major phenotypes: 1-1, 2-1 and 2-2. These three phenotypes have different structural and functional properties. HP 1-1 is a much better antioxidant than HP 2-2 (Gueye et al. 2006). On the other hand, HP 2-2 is more angiogenic than other phenotypes (Cid et al. 1983; Cockerill et al. 1995). HP 2-1 has functional properties at an intermediate level. Most attention has been paid in determining haptoglobin phenotype as a genetic fingerprint used in forensic medicine. More recently, several functional differences between haptoglobin phenotypes have been demonstrated that appear to have important biological and clinical sequences. Haptoglobin polymorphism is associated with the prevalence and clinical evolution of many inflammatory diseases, including infections, atherosclerosis and autoimmune disorders. These effects are explained by a phenotype-dependent modulation of oxidative stress and prostaglandin synthesis. Recent evidence is growing that haptoglobin is involved in the immune response as well. The strong genetic pressure favoring the 2-2 phenotype suggests an important role of haptoglobin in human pathology (Langlois and Delanghe 1996).

Haptoglobin has also been identified as a strong angiogenic agent, activating endothelial cell growth and differentiation. This function was shown to be phenotype dependent, as the HP 2-2 phenotype has been found to be more angiogenic than the other phenotypes (Cid 1993). The anti-oxidative capacity of body fluids is less efficient in HP 2-2 individuals. The poor prognosis of our HP 2-2 cervical cancer patients in our discovery set could be exerted via this haptoglobin function, since angiogenesis is well known to be involved tumor growth, proliferation, and metastasis (de Castro et al. 2006).

The allele frequencies of GC*1 and GC*2 are 0.5200 and 0.4800 in cervical cancer patients and in controls it is 0.6900 and 0.3100 res-pectively. In this marker there is a decreased frequency of GC*1 and increased frequency of GC*2 of diseased patients when compared with controls. The chi- square test for homogeneity was found to be non-significant in both controls and patients (χ^2 = 0.0165; χ^2 = 0.0739 respectively).The inter group heterogeneity was also found to be non-significant (χ^2 = 5.7136; d.f = 2; 0.10 > p > 0.05). Thus, both the examined groups were in Hardy-Weinberg equilibrium indicating no association between cervical cancer and this protein marker. But it may be pointed out here that sub typing was not done in the present study and that most of the studies reported GC associations with diseases so far have been found with GC 1F phenotype.

On the other hand, the remaining markers transferrin, albumin and caeruloplasmin show monomorphism with frequency of 1.0000 in both cervical and controls. Therefore, no association was observed between cervical cancer and TF, ALB and CP systems.

The odds ratio and relative risks for each genotype versus the other two are shown in Table 3. Homozygotes 2-2 phenotype patients were at an increased risk of cervical cancer, with an overall odds ratio of 3.30 (95% C.I: 1.21-9.14, p = 0.0088) by the method of Der Simonian and Laird. Heterozygotes (2-1) were at reduced risk of cervical cancer, with an overall odds ratio of 0.36 (95% C.I: 0.13-0.98, p = 0.0259).

In the present study, Risk estimates show a significant association of HP 2-2 phenotypes with cervical cancer (RR = 1.41) individuals. The result shows an increased risk of 40% more, indicating that HP 2-2 individuals are more likely to get the disease when compared with the other phenotypes of the haptoglobin system.

Table 3: Test of association, relative risk and odds ratio estimates of HP phenotypes in disease and control groups

HP phenotypes	*Control (n)*	*Cervical cancer*				
		(n)	*RR*	*OR*	*95% CI*	*χ^2 values*
1-1 vs 2-1 + 2-2	2	0	-	0.00	(0.00-4.11)	2.04
2-1 vs 1-1 + 2-2	19	9	0.47	0.36	(0.13-0.98)	4.96*
2-2 vs 1-1 + 2-1	29	41	1.41	3.30	(1.21-9.14)	6.86**

RR-Relative Risk; *p<0.05, **p<0.01

CONCLUSION

To summarize, out of the five genetic markers studied, only one genetic marker namely, haptoglobin has shown significant association with cervical cancer. The study population showed the predominant occurrence of HP 2-2 phenotype in cervical cancer patients. The haptoglobin phenotypes can aid in predicting or preventing a disorder and in determining a more precise prognosis and better treatment. Because of small sample size, the conclusions drawn can at best be tentative and therefore an extensive work is needed for better understanding of the role of haptoglobin in the pathophysiology of cervical cancer.

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