Research Article

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ASSOCIATION OF VARIOUS GENETIC MARKERS IN PSORIASIS AND VITILIGO

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Abstract: Psoriasis and Vitiligo are the skin disorders which are mediated by immunologically and inherited genetically. These are most predominant in the cases where the immune system sends faulty signals and by which the body produces an immune response against some of its own cells. Psoriasis occurs when skin cells grow too quickly. Where as in Vitiligo the immune system destroys pigmented making cells called melanocytes. This results in lesions formation and white patches of skin on different parts of the body. The present study undergone to determine whether the biochemical genetic marker phenotypes are predictive of Psoriasis and Vitiligo. Using biochemical techniques the association between Psoriasis and Vitiligo with Red cell enzymes like ESD, SOD, ACP1, GLO1 and Plasma proteins like HP, CP, ALB, GC, and TF have been extensively studied on 40 individuals of each disorder and 80 healthy controls. The results were compared with the healthy population series investigated for the same genetic markers. There was no significant association with any of the marker systems.

Keywords: Genetic markers, Psoriasis, Vitiligo, Red Cell Enzymes, Plasma Proteins.

INTRODUCTION

Psoriasis: It is an immune-mediated disease that affects the skin [1]. It is not contagious and can be inherited. It occurs when the immune system mistakes a normal skin cell for a pathogen and sends out faulty signals and by which the body produces an immune response against some of its own cell [2]. In this, the person's immune system and genes play key roles. In studying the immune system, it was discovered that when a person has psoriasis, the T cells - a type of white blood cell that fights unwanted invaders such as bacteria and viruses- mistakenly trigger a reaction in the skin cells. This is why psoriasis is referred to as a "T cell-mediated disease" [3]. This reaction activates a series of events, causing skin cells to form in days rather than weeks. The body does not shed these excess new skin cells, so the cells pile up on the surface of the skin and lesions form. There are five types of psoriasis: plaque, guttate, inverse, pustular, and erythrodermic. It is seen worldwide in all races, both sexes and can be seen in people of any age [4]. Signs and symptoms include patches of raised red skin with thick silvery scales. The affected skin may be red and scaly or have pustules, depending on the type of psoriasis the individual has. There is currently no cure, but various treatments can help to control the symptoms.

Vitiligo: It is an acquired, idiopathic disease that causes depigmentation of sections of skin [5]. It occurs when absence of melanocytes- the cells responsible for skin pigmentation- die or unable to function is the key element of pathogenesis of Vitiligo. The etiology of vitiligo is still unknown. But, due to genetic and neurological factors, auto immunity, oxidative stress, toxic metabolites, and lack of melanocytes growth factors might contribute for precipitating the disease in susceptible people [6]. The most notable symptom of Vitiligo is depigmentation of patches of skin that occurs on the extremities. Hair in these areas is normally white or gray, and the skin tends to sunburn a lot easier. Although patches are initially small, they often enlarge and change shape. It is equally common in men and women. It can appear at any age but 50 per cent of patients are under 20 when it first appears. Symptoms involve the physical appearance as well as its psychological impact. Vitiligo appears to be not only polygenic but also multifactorial [7]. These means that there are other factors that probably work in combination and cause the disease. These factors may include the body's immune system that may destroy melanocytes; abnormally functioning nerve cells that may produce toxic substances that injure melanocytes; production of toxic by-products that could injure and destroy melanocytes while pigment is forming; and environmental factors, such as infections or damage to the skin [8].

A) Psoriasis

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B) Vitiligo

Figure.1: Images of Psoriasis and Vitiligo Symptoms

MATERIALS AND METHODS

Collection of blood samples:

The blood samples were collected from The King George Hospital, Visakhapatnam. The sample size is 40 of psoriatic patients & 40 of Vitiligo patients of both males & females and 80 healthy controls matched for both age and sex and put those in thermos flask containing ice, within a few hours of collection. 5 ml of intravenous samples were collected into sterilized test tubes containing 15% EDTA as an anti-coagulant. The blood specimens were centrifuged for 10 minutes at 1000rpm and the supernatant plasma was separated into small plastic labelled vials and stored at -20 c until analysis for plasma proteins. Buffy coat was collected from the sample and genomic DNA was isolated. The remaining packed volume of red blood cells was washed for three times with saline (0.9%) to get clear red blood cells. The packed red cells were stored in labeled test tubes at 4^{o} c for analyzing red cell enzymes.

Hemolysate preparation:

The packed red cells were then haemolysed with equal volumes of distilled water by vortexing for few minutes. The red cell hemolysates were washed with carbon tetra chloride (0.5-1.0) and mixed with the help of cyclomixer. Then it is spun at 3000rpm for 20minutes. The clear red cell lysates were transferred into labeled vials and stored at -20 c until further use.

Biochemical methods:

For Red cell Enzymes: The technique of horizontal gel electrophoresis [9] was used for the typing of red cell enzyme systems Esterase-D (ESD), Super Oxide Dismutase (SOD), Acid Phosphatase (ACP1), and Glyoxalase 1 (GLO1) included in the present study. The technique performs separation of a particular enzyme in a supporting such as Agarose or Starch with slight modifications using specific buffer & specific biochemical staining methods.

For Plasma Proteins: Polyacrylamide gel electrophoresis [10] [Disc gel electrophoresis –DISC PAGE] was employed in the present study to screen the plasma proteins for haptoglobin (HP), tranferrin (Tf), albumin (ALB) and ceruloplasmin (CP) and group specific component (GC) systems.

After electrophoresis, the staining mixture was applied on to the surface of the gel and incubated. After incubation washed in distilled water/ the gel blotted for free from excess stain. The gel plate was then observed under Ultraviolet light. Based on their mobility in the gel types of phenotypes were identified as fast moving, medium and slow moving using their banding patterns respectively.

Statistical Analysis:

The allele frequencies were estimated by maximum likelihood method and statistical significance of differences between diseased patients and controls were tested by X^2 test.

$$X^2=\sum (O-E)^2/E$$

Where $\sum$ stands for the summation, E= Expected number, O= Observed Number

RESUTLS AND DISCUSSION

Table.1: Distribution of Enzyme Phenotypes in Psoriatic, Vitiligo patients and controls

System	Phenotype	Psoriatic patients (% distribution in brackets)		Vitiligo patients (% distribution in brackets)		Controls (% distribution in brackets)	
		Observed	Expected	Observed	Expected	Observed	Expected
ESD	1-1	22(55)	24.05	17(42.5)	18.22	31(39)	36.45
	2-1	18(45)	13.90	20(50)	17.55	46(58)	35.1
	2-2	0(0)	2.05	3(7.5)	4.22	3(4)	8.45
TOTAL		40	40	40	40	80	80
		X^2-3.43; df=1; (0.10>p>0.05)		X^2=0.7794 df=1 (0.50>p>0.3)		X^2-7.71; df=1; (0.01>p>0.001)	
SOD	1-1	40(100)	-	40(100)	-	80(100)	-
ACP	1-1	5(12.5)	6.4	5(12.5)	4.22	4(5)	7.2
	2-1	22(55)	19.2	16(40)	17.5	40(50)	33.6
	2-2	13(32.5)	14.4	19(47.5)	18.22	36(45)	39.2
TOTAL		40	40	40	40	80	80
		X^2=0.85; df=1 (0.50>p>0.30)		X^2=0.3118; df=1 (0.70>p>0.5)		X^2=2.902; df=1; (0.10>p>0.05)	
GLO	1-1	7(17.5)	7.225	5(12.5)	3.6	12(15)	14.861
	2-1	20(50)	19.55	14(35)	16.8	45(56.25)	39.238
	2-2	13(32.5)	13.225	21(52.5)	19.6	23(28.75)	
TOTAL		X^2=0.021 df=1 (0.90>p>0.8)		X^2=1.10; df=1; (0.30>p>0.20)		X^2=1.720; df=1; (0.20>p>0.10)	

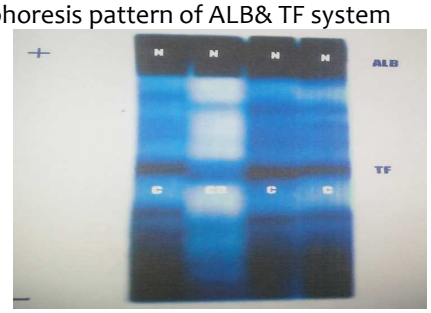
Table 2: Distribution of Plasma Protein Phenotypes in Psoriatic, Vitiligo patients and controls

System	Phenotype	Psoriatic patients (% distribution in brackets)		Vitiligo patients (% distribution in brackets)		Controls (% distribution in brackets)	
		Observed	Expected	Observed	Expected	Observed	Expected
HP	1-1	2(5)	1.086	0(0)	0.51	0(0)	0.8
	2-1	13(32.5)	13.38	9(22.5)	7.98	16(20)	14.4
	2-2	25(62.5)	24.80	31(77.5)	31.5	64(80)	64.8
	TOTAL	40 40 X^2=0.03;df=1 (0.90>p>0.80)		40 40 X^2=0.648;df=1 (0.80>p>0.70)		80 80 X^2=0.979;df=1; (0.50>p>0.30)	
GC	1-1	12(30)	12.1	15(37.5)	14.1	40(50)	41.37
	2-1	20(50)	19.8	18(45)	19.2	35(43.75)	32.32
	2-2	8(20)	8.1	7(17.5)	6.4	5(6.25)	6.31
	TOTAL	40 40 X^2=0.00;df=1 (0.95>p>0.90)		40 40 X^2=0.156;df=1 (0.70>p>0.50)		80 80 X^2=0.539;df=1; (0.50>p>0.30)	
TF	C	40	-	40	-	80	-
ALB	N	40	-	40	-	80	-
CP	B	40	-	40	-	80	-

Table 3: Distribution of Allele Frequencies in Psoriatic, Vitiligo patients and controls

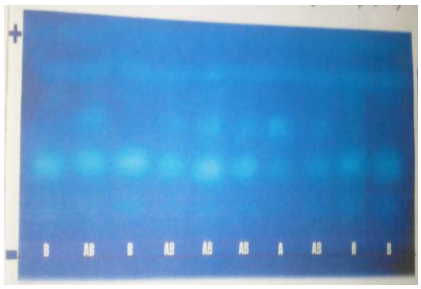
System		Psoriasis	Vitiligo	Intergroup Heterogeneity Psoriasis/Vitiligo	D.F
ESD	1	0.7750 ±0.0660	0.675 ±0.0740	3.92/0.528	
	2	0.2250 ±0.0660	0.325 ±0.0740		2
SOD	1	1.0000 ±0.0000	1.0000 ±0.0000	-	-
ACP	1	0.400 ±0.0775	0.325 ±0.0740	2.16/1.746	
	2	0.600 ±0.0775	0.675 ±0.0740		2
GLO	1	0.425 ±0.0781	0.300 ±0.0724	0.196/4.32	
	2	0.575 ±0.0781	0.700 ±0.0724		2
HP	1	0.2125 ±0.0646	0.1125 ±0.0499	4.02/0.072	
	2	0.7875 ±0.0646	0.8875 ±0.0499		2
GC	1	0.5500 ±0.0786	0.600 ±0.0774		
	2	0.4500 ±0.0786	0.400 ±0.0774	4.5/2.328	2
TF	C	1.0000 ±0.0000	1.0000 ±0.0000		
ALB	N	1.0000 ±0.0000	1.0000 ±0.0000		
CP	B	1.0000 ±0.0000	1.0000 ±0.0000		

Electrophoresis pattern of ALB& TF system



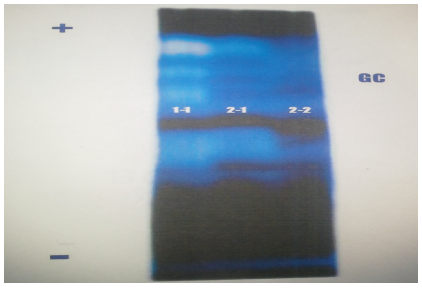
A

Electrophoresis pattern of Red Cell Acid Phosphatase (ACO 1)



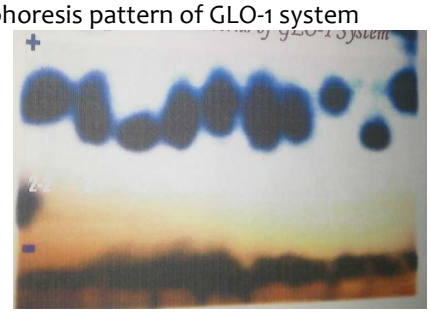
C

Electrophoresis pattern of Group Specific Component (GC) system

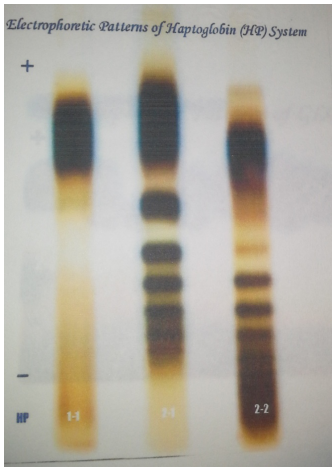


B

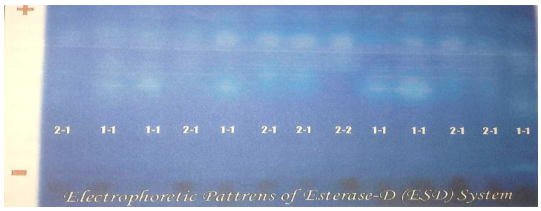
Electrophoresis pattern of GLO-1 system



D



E



F

Figure.2: Images Showing Electrophoretic patterns of Genetic markers A) ALB & TF SYSTEM B) GC SYSTEM B) ACP1 D) GLO1 Systems E) HP System F) ESD System.

The enzyme and protein markers such as ESD, SOD, ACP1, GLO1, HP, CP, TF, ALB and GC were analyzed for determining their allelic variations. This study shows the distribution of these marker phenotypes in control individual's percentage in different electrophoretic patterns with increased or decreased frequencies in Psoriatic and Vitiligo Patients as in table 1 and 2. Where as in distribution of allele frequencies with its chi-square test values, intergroup heterogeneity found to be not significant [Table 3]. No variation is seen in the markers such as TF, ALB and CP. They are similar in both controls and diseased and the phenotypes showing normal C, N and B type respectively.

CONCLUSION

In the present study, sample size is 40 individuals of psoriasis and Vitiligo diseased patients respectively both male and females and 80 healthy controls matched for both age and sex samples were considered. It undergone through the biochemical approach to identify the association of various red cell and plasma protein markers based on biochemical studies in psoriasis and vitiligo patients. The allele frequencies of these described markers in controls and in diseased patients respectively. The chi-square test for diseased patients found to be the values which are not significant. This shows much deviation in the X^2 values individually in diseased patients and controls. The X^2 test value with degrees of freedom 2 for heterogeneity was found to be not significant. Thus, it represents no association between diseased patients and genetic markers such as red cell enzymes (ESD, SOD, ACP1, and GLO1) and plasma proteins. (HP, CP, GC, ALB, TF).

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