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Original Research Article

A Study on the LH-specificity in Patients with Type-2 Diabetes Mellitus of North India

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Abstract

Background: Diabetes mellitus is a group of metabolic diseases and has become epidemic worldwide. The present study was conducted to find the LH status of patients with diabetes mellitus with the anti-LH lectin *Erythrina lithosperma*.
Methods: A confirmed 263 cases with type 2 diabetes mellitus and 251 controls were enrolled in the study. ABO typing was done by normal serological procedure. LH typing was done with appropriate procedure. ABO blood group and LH specificity were compared in diabetic and control groups.
Results: Patients with diabetes mellitus had higher frequency (41.06%) in LH-negative type than controls (14.34%), showing highly significant differences ($p<0.001$) between them. Statistically significant differences ($p<0.05$-0.001) were noted in blood group B, AB and O between these two sets of population.
Conclusion: It might be concluded from the findings of the present study that diabetic patients had significantly more LH negative type as compared to controls, because the erythrocyte membrane in diabetic patients were always in hyperglycemic environment and membrane receptors were saturated with glucose molecules, leaving less availability of receptors to bind with anti-LH lectin *Erythrina lithosperma.*

Keywords: ABO blood groups, Diabetes mellitus, *Erythrina lithosperma*, LH Specificity

1. Introduction

Diabetes Mellitus (DM) is described as metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion or insulin action or both [1]. It has become now a disease of major concern globally and is a leading cause of death in most countries [2]. In 2013, the International Diabetes Federation (IDF) estimated that 382 million people would be diabetes worldwide, and by 2035, it was predicted to rise to 592 million. 80% live in low- and middle-income countries, and of the total, more than 60% live in Asia. According to Diabetes Atlas published by the International Diabetes Federation (IDF), there were estimated 40 million persons with diabetes in India in 2007 and this number was predicted to rise to almost 70 million people by 2025. The countries with the largest number of diabetic people would be India, China and USA by 2030 [3]. Major increase in the prevalence of diabetes occurred in developing countries due to rapid and ongoing socioeconomic transition and would likely lead to further rises [3]. The prevalence of both type 1 and type 2 diabetes (T2DM) has increased significantly during recent decades. T2DM, being much more common [3], has been the main driver for the increase in global diabetes prevalence and, therefore, should be focused more.

Lectins are carbohydrates binding proteins which recognize and interact with specific monosaccharide or oligosaccharides units present on the erythrocyte's membrane. Shrivastava *et al*. described new cell membrane specificity in the seed extracts of *Erythrina lithosperma*, a plant belonging to the family Leguminosae and named the specificity as LH types. The name LH was given to this specificity in the memory of Ludwik Hirszfeld, the Polish serologist. Further studies relating to the immunochemical properties of the anti-LH lectin, its genetics and its distribution in various populations have been reported by Shrivastava *et al*. [11], Sehjpal and Shrivastava [9,10], Reddy *et al*.[8], Kaur [4], Koley and Sandhu[13,14,15] Koley *et al*. [5-7,12,16-21,23-24,27] and Koley and Shrivastava [22,25,26,28]. Lectin isolated from the seeds of *Erythrina lithosperma*, called as anti-LH. The anti-LH lectin interacts with erythrocytes by either clumping them firmly or weakly agglutinating them. The former type of reaction is called LH-positive and the latter LH-negative. Difference between the reaction pattern of LH positive and LH negative might

depend upon the distribution of lectin binding receptors present on erythrocyte membranes, which are similar or identical structurally to those carbohydrate receptors. Thus it was hypothesized that in variously glycosylated red blood cells from patients with type 2 diabetes mellitus there should be fewer receptors available on the cell surface to bind the lectin molecules and, its consequences, there should be more LH-negative individuals among patients with type 2 diabetes mellitus than the normal population.

2. Material and Methods

2.1 Samples

A total of 263 confirmed cases of type 2 diabetes mellitus were obtained from Guru Nanak Dev Hospital and Dr. Puneet Arora Diabetic and Research Centre, Green Avenue, Amritsar, Punjab, India. The samples comprised of both males and females. All diabetic patients were aged 40 and above. A total of 251 normal healthy individuals sampled randomly from this area acted as control group. Since sex differences are not known to exist in the LH system, the samples collected from both males and females were pooled for all analyses. The informed consent was taken from each diabetic patient who participated in the study. This study was approved by Institutional Ethical Committee.

2.2 ABO typing

The finger was sterilized and pricked with sterilized lancet. The blood was collected in 0.85% NaCl (physiological saline) in test tube. RBCs pellet was washed thrice with physiological saline by centrifuging it at 2500-3000 rpm. 2% RBCs suspension prepared in physiological saline. ABO typing was done by normal serological method using 2% RBCs suspension and with anti-A, anti-B and anti-D.

2.3 The Anti-LH lectin

The LH-typing was done with anti-LH lectins prepared from the seeds of *Erythrina lithosperma* as described by Shrivastava *et al* [11, 24]. The seeds of *Erythrina lithospermia* were obtained from Botanical survey of India, Kolkata.

2.4 Preparation of Anti-LH lectin

The anti-LH lectin from seed extracts of Erythrina lithosperma is prepared in normal saline. The seeds are ground to a fine powder and mixed with normal saline in the ratio 1:9. The mixture I then allowed to stand at ambient temperature for four hours, with occasional stirring. After this period the slurry is centrifuged at 3000-4000 rpm for 30 minutes. The clear supernatant is subsequently separated and stored under refrigeration with sodium azide added to it in the ratio of 1:10000 parts as preservative.

2.5 LH typing

For the LH typing, a single drop of anti-LH lectin (*Erythrina lithosperma*) from a Pasteur pipet was added to four drops of 2% suspension of red blood cells in normal saline in a serological test tube measuring 15mm x 15 mm. The mixture was then centrifuged for 20 seconds at 2500rpm. A cell button was formed in all cases but in some brisk manual shaking of the tube resulted in dispersal of the button into a large number of minor agglutinates whereas in other similar shaking left the cell button intact. The former type of reaction was called LH- negative and the latter type as LH-positive. The results of the tube test might be checked by the method of titration. With LH-positive cells, the titre ranged between 32-64 and with the LH-negative cells between 8-16. [23]

2.6 Statistical analysis

Frequency distribution of ABO and LH typing were made followed by chi-square test for the correlation of LH typing with ABO blood type. Data were analyzed using SPSS (Statistical Package for Social Science) version 20.0. A 5% level of probability was used to indicate statistical significance.

3. Results

The phenotypic distribution of the LH-types and allele frequencies in patients with type 2 diabetes mellitus and controls are given in table 1. Patients have higher frequency (41.06%) in LH-negative type than controls (14.34%). The difference between the patients and controls in regards to the LH-types was highly significant ($p<0.001$). In patients, the allele frequency of LH-positive type was (0.77) and in controls it was (0.93). Both these populations was in Hardy-Weinberg equilibrium

Table 2 showed the distribution of the ABO blood groups along with their allele frequencies in patients and controls. Patients have slightly higher frequencies in group B (37.64%) and group O (29.27%) than their control counterparts, while group AB were less frequent in patients (9.125%) than controls and group A were equally distributed in both patients (23.95%) and controls (23.59%). There existed no significant difference ($p>0.05$) among them. Controls have higher A (0.218) and B (0.286) allele frequencies than patients while the allele frequency of O (0.558) was higher in patients than controls. Both these populations were in Hardy-Weinberg equilibrium.

Table 1: Distribution of the LH types and allele frequencies in patients with type 2 diabetes mellitus and controls

Samples	N	Phenotype frequencies			Allele frequencies		$X^2_{(1)}$	p
			LH^+	LH^-	LH^+	LH^-		
Patients	263	n	155	108	0.77	0.23	45.74	p<0.001
		%	58.93%	41.06%				
Controls	251	n	215	36	0.93	0.07		
		%	85.65%	14.34%				

The ABO group wise distribution of LH-types in patients and controls were given in table 3. Patients had higher frequencies in A/LH$^-$ (7.98%), B/LH$^-$ (23.57%) and AB/LH$^-$ (7.22%) combinations than controls.

Table 4 showed the distribution of the LH-types with blood group A in patients and controls. There were almost equal distribution of LH-negative individuals in patients (7.98%) and controls (5.97%) showing no significant difference ($p>0.05$) among them, although both the populations were in Hardy-Weinberg equilibrium.

The distribution of the LH-types with blood group B in patients and controls were given in table 5. More LH-negative type was reported in patients (23.57%) than controls (3.187%) and the difference was statistically significant ($p<0.001$) However, patients and controls were in Hardy-Weinberg equilibrium.

Table 2: Distribution of the ABO types and allele frequencies in patients with type 2 diabetes mellitus and controls

			Phenotypic frequencies				Allele frequencies			$X^2_{(3)}$	p
Samples	**N**		**O**	**A**	**B**	**AB**	**A**	**B**	**O**		
Patients	263	n	77	63	99	24	0.162	0.273	0.558	1.319	p>0.05
		%	29.27	23.95	37.64	9.12					
Controls	251	n	71	59	89	32	0.218	0.286	0.520		
		%	26.99	23.59	33.84	12.74					

Table 6 showed the distribution of the LH-types with blood group O in patients and controls. The patients had more LH-negative individuals (2.28%) than controls (0.39%), showing statistically significant difference ($p<0.001$) between them. However, patients and controls were in Hardy-Weinberg equilibrium.

The distribution of the LH-types with blood group AB in patients and controls was given in table 7. The patients (7.224%) had more LH-negative individuals than controls (4.78%) and the difference was statistically significant ($p<0.001$). Never the less both these population were in Hardy-Weinberg equilibrium.

Table 3: ABO group-wise distribution of the LH types in patients with type 2 Diabetes mellitus and Controls

Samples	**N**	**O**				**A**			**B**			**AB**		
Patients	263	n	n	LH$^+$	LH$^-$	n	LH+	LH-		LH+	LH-	n	LH+	LH-
			77	71	06	63	42	21	99	37	62	24	05	19
		%	29.27	26.99	2.28	23.95	15.96	7.98	37.64	14.06	23.57	9.125	1.90	7.22
Controls	251	n	71	70	1	59	44	15	89	81	08	32	20	12
		%	26.99	27.88	0.39	23.50	17.52	5.97	35.45	32.27	3.187	12.74	7.968	4.78

Table 4: Distribution of the LH types in patients with type 2 diabetes mellitus and controls with blood group A

Samples	**N**	**Obs**	**LH$^+$**	**LH$^-$**	**$X^2_{(1)}$**	**p**
Patients	63	n	42	21	0.913	p>0.05
		%	15.96%	7.98%		
Controls	59	n	44	15		
		%	17.52%	5.97%		

Table 5: Distribution of the LH types in patients with type 2 Diabetes mellitus and controls with Blood group B

Samples	**N**	**Obs**	**LH$^+$**	**LH$^-$**	**$X^2_{(1)}$**	**p**
Patients	99	n	37	62	57.68	p<0.001
		%	14.06%	23.57%		
Controls	89	n	81	8		
		%	32.27%	3.187%		

Table 6: Distribution of the LH types in patients with type 2 diabetes mellitus and controls with blood group O

Samples	**N**	**Obs**	**LH$^+$**	**LH$^-$**	**$X^2_{(1)}$**	**p**
Patients	77	n	71	06	3.3393	p<0.001
		%	26.99%	2.28%		
Controls	71	n	70	01		
		%	27.88%	0.39%		

Table 7: Distribution of the LH types in patients with type 2 Diabetes mellitus and controls with Blood group AB

Samples	**N**	**Obs**	**LH$^+$**	**LH$^-$**	**$X^2_{(1)}$**	**p**
Patients	24	n	05	19	9.628	p<0.001
		%	1.90%	7.224%		
Controls	32	n	20	12		
		%	7.968%	4.78%		

4. Discussion

The purpose of the study was to find out the difference in the LH specificity of patients with type 2 diabetes mellitus and controls. This hypothesis was generated on the assumption that in diabetic patients persistent hyperglycemia either due to insufficient production of insulin by pancreas or improper utilization of the glucose, erythrocytes remain in hyperglycemic environment throughout their life span which leads to significant changes in erythrocyte aggregation and deformability, It has also been reported that the cytoskeleton proteins of the erythrocytes from diabetic patients are heavily glycosylated and that spectrin is oxidatively damaged [29]; also several lipids (free cholesterol, sphingomeyelin and phosphatidylcholine) on the outer surface of the phospholipid bilayer were significantly decreased [30,31]. Because of excessive in vivo glycosylation on the cell membrane of erythrocyte, there would be less number of attachment sites or receptors available to the carbohydrate-rich reactive component of the anti-LH lectin than would normal erythrocytes. Therefore, in patients with type 2 diabetes mellitus there should be more LH-negative individuals than in the normal population. The results of the present study indicated statistically significant difference in the LH specificity of patients with type 2 diabetes mellitus as compare to controls i.e. patients with type 2 diabetes mellitus were overwhelmingly LH-negative ($p<0.001$) as compare to controls, and also that these differences were particularly noticeable in patients with the blood group B, AB and O. A significant preponderance of LH-negative type in diabetic patients, with blood group B, AB and O allows to conclude that the LH system has more than a chance association with diabetes mellitus. The results of the previous studies indicated that as against normal individuals the patients with diabetes mellitus were LH negative ($p<0.001$) and also that this difference was particularly noticed in patients with blood group A and B. The findings of the present study followed the finding of previous studies [5, 11, 20]. One striking finding of the present study created controversy reporting that the negative status in O individuals (6 in patients and 1 in control). Earlier it was reported that individual with O blood type were invariably LH-positive [4-10,11,13,15,16,20,25,26]. The question remained unanswered. Further studies covering vast sample size might solve the controversy.

5. Conclusion

Thus the present study concluded that there are more LH-negative individuals in diabetic patients compare to controls. Because erythrocytes in diabetic patients are remains in hyperglycemic environment and receptors on cell membrane are saturated with glucose molecules and theses receptors are also the binding sites for lectin molecules, due to unavailability of carbohydrate binding sites for the anti-LH lectin cannot bind thus more LH-negative individuals.

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