IMMUNOGLOBULIN LEVELS OF VITILIGO PATIENTS

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ABSTRACT

In the present study, the serum immunoglobulin profiles of vitiligo patients were compared with that of cohort control and evaluated the correlation between immunoglobulin level with their socioeconomic factors and nutritional status. Thirty vitiligo patients were recruited randomly from the Department of Dermatology and Venereology, Bangabandhu Sheikh Mujib Medical University Hospital, Dhaka, Bangladesh for this study. Thirty healthy individuals as control group matched by age, sex, education and socioeconomic factors to the patient group were selected. Serum immunoglobulin concentrations were determined by turbidimetry method using immunoglobulin kit. The concentration of IgG and IgA decreased significantly ($P<0.05$), but the change of IgM was not significant. Socioeconomic data revealed that most of the patients were young and female. Moreover statistical analysis revealed that there was significant correlation between immunoglobulin (IgG and IgA only) concentrations and BMI and number of depigmented patches with IgG concentrations. Finally it can be concluded that the change of serum immunoglobulin concentration in vitiligo patients could be due to the disease condition as pathomechanism suggested the aberrations in cellular immunity. But study with larger number of population is required for further evaluation of the relationship between the immune response and disease state to confirm these findings.

Keywords: Vitiligo, immunoglobulins, socioeconomic factors.

INTRODUCTION

Vitiligo is an acquired leukoderma that results from the loss of epidermal melanocytes, and characterized by macules and patches of depigmented skin. Vitiligo usually occurs in localized, generalized, or segmental patterns with rapid progression or remains stationary. The worldwide prevalence of vitiligo is estimated to range between 0.1% and 8.8% (Arican and Kurutas, 2008) and onset of the disease is before 20 years of age in 50% of cases (Majumder *et al*., 1993). Vitiligo is not believed to be sex-linked, but 6-38% of patients have a positive family history (Ortonne *et al*., 1983). Though vitiligo is not considered as a major disease, but psychological and social impact can not be underestimated (Kent and Al Abadie, 1996, 1996).

Despite much research, the etiology of vitiligo and the causes of melanocyte death are not clear. Possible pathogenic mechanisms for vitiligo included immunological, neural, and biochemical, but complete explanation is not well established (Dell'anna *et al*., 2003; Arýcan, 2004). Segmental vitiligo can be correlated with neurological theory whereas autoimmune mechanism can be accounted for generalized (non-segmental) form of the disorder (Taeib, 2000).

Previous studies suggested the involvement of both cellular and humoral immunity in the pathogenesis of vitiligo (Ogg *et al*., 1998). The autoimmune hypothesis suggests the development of antibodies against melanocyte surface antigens (Kovacs, 1998). Abnormal expression of the MHC class II antigen HLA-DR and increased expression of intercellular adhesion molecule-1 (ICAM-1) have been found in perilesional melanocytes in vitiligo compared with those pigment cells from normal skin (Al Badri *et al*., 1993; Van den Wijngaard *et al*., 2000). So expression of these molecules by melanocytes might affect the abnormal cellular immune response against vitiligo. Moreover autoimmunity might contribute for the self-destruction of pigment cells and this might then enhance the damage to melanocytes. Gauthier *et al*., recently proposed the melanocytorrhagy hypothesis, which is based on an *in vivo* observation of melanocyte detachment from the basal layer, followed by transepidermal migration, which in turn triggers

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melanocyte death (Gauthier *et al*., 2003). According to Alkhateeb *et al*., a higher frequency of autoimmune thyroid disease, Addison's disease, systemic lupus erythematosus and pernicious anaemia were found, among them about 30% of patients being affected with at least one additional autoimmune disorder (Alkhateeb *et al*., 2003). Moreover a significant percentage of vitiligo patients were found with circulatory antibodies to melanocytes and the presence of these antibodies were related to the extent of the disease (Farrokhi *et al*., 2005 and Naughton *et al*., 1986).

In a previous study by Mittal *et al*., peripheral T-lymphocyte count in vitiligo patients were found to be lower than normal and serum immunoglobulin concentrations were abnormal but the difference in immunoglobulin levels was statistically insignificant (Mittal *et al*., 1994). An increase of IgE count was found in 22% of vitiligo patients (Perfetti *et al*., 1991). Patients with vitiligo and simultaneous atopy presented lower IgE and higher IgA values compared to a reference group of patients with atopy alone (Chatain *et al*., 1994). A few other studies described the quantitative status of humoral and cellular immunity of vitiligo patients. But for more clear resolution of the facts, the current research was focused on to evaluate the extent of involvement of these immune responses in vitiligo. Moreover no research has yet been conducted on immunoglobulin profile in Bangladeshi vitiligo patients. That is why we investigated the role of immune systems by measuring the serum levels of three immunoglobulins IgG, IgM and IgA in vitiligo patients and in healthy controls.

MATERIALS AND METHODS

Subjects and study design

This was a type of case control study. Thirty vitiligo patients were recruited, who attended out-patient Department of Dermatology and Venereology, Bangabandhu Sheikh Mujib Medical University Hospital, Dhaka, Bangladesh, during the period of September' 2007 to February' 2008. The patients comprised of 10 males and 20 females, age ranging from 14 to 50 years with mean BMI of 22.52 (± 3.07) kg/m^2. The cases showing white patches due to secondary causes were excluded from this study. A complete clinical examination was performed, and the site and pattern of the lesions were noted. The control group included 30 healthy individuals matched by age, sex, education and socioeconomic status to the patient group, with no previous history of any autoimmune or immunomodulatory disorders or any medical disease that can affect the immune system.

Different investigations were carried out for all patients like hemoglobin level, total and differential leukocyte counts, erythrocyte sedimentation rate, peripheral smear, blood glucose level, RBS, Wood's lamp examination, triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), and antithyroid antibodies. Exclusion criteria were patients with previous diseases that can affect immunity, e.g. rheumatic fever, rheumatoid arthritis, liver diseases, viral hepatitis, steatorrhoea, renal diseases, neoplastic condition, myocardial infarction etc.; patients who received any other medication such as oral contraceptives, non-steroidal anti-inflammatory drugs, corticosteroids, immunosuppressing agents, or had ECT during the preceding 6 months.

The study subjects were briefed about the purpose of the study and written consent was taken from each of them. Each of the subjects filled up a questionnaire form that contains personal information, socio-economic data, history and current status of illness, family history etc. The forms of the patients who had no formal education were filled out with the help of an investigator. Ethical approval was obtained from the Ethical Review Committee of Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU).

Blood collection

Five mL venous blood sample was collected from the antecubital vein of each of the vitiligo patients and healthy volunteers in a metal-free sterile tube, between 8 to 9 am after an overnight fasting in a dust free environment. Samples with signs of hemolysis were discarded. The blood was then allowed to clot at room temperature for 30 minutes and centrifuged for 15 minutes at 3000 rpm to extract the serum. The serum was aliquoted into eppendorf tubes and stored at −80°C for analysis of immunoglobulins. Blood collection and serum separation were carried out in a dust-free environment (Khanam *et al*., 2008).

Immunoglobulin Profiling

The serum immunoglobulin (IgG, IgA, and IgM) levels in both patients and controls were determined by turbidimetry method described by Khanam *et al* using an immunoglobulin kit (Quantia, India) (Khanam *et al*., 2008). In this method, anti-human antibodies for IgG, IgA and IgM were mixed with serum samples containing IgG, IgA, and IgM that formed insoluble antigen-antibody complexes. These complexes caused an absorbance change depending upon the immunoglobulin concentrations that was quantified by a calibrator.

Calibrator standards of different immunoglobulins were prepared by diluting the reference standards with normal saline to get the desired concentration ranges (40-800 mg/dL for IgG, 6-144 mg/dL for IgA and 4-80 mg/dL for IgM).

5µL of all the calibrator standards were pipetted into marked wells of each of the microtitre plate. Similarly the 5µL of diluted serum (1:10 with normal saline) samples

Table 1: Socioeconomic Status of vitiligo patients (n = 30) and controls (n = 30).

Parameter	Patients			Control			*P*-value
	n	%	Mean ± SD	n	%	Mean ± SD	
Age (years)							
11 – 20	5	16.7	30.27 ± 11.09	5	16.7	32.27 ± 11.08	*0.555**
21 – 30	15	50.0		15	50.0		
31 – 40	4	13.3		4	13.3		
41 – 50	4	13.3		4	13.3		
51 – 60	2	6.7		2	6.7		
Area of Residence							
Rural	26	86.67	ND	28	93.33	ND	*0.584***
Urban	3	10		1	3.33		
Suburban	1	3.33		1	3.33		
Education							
Illiterate/Read only	3	10.00	ND	6	20.00	ND	*0.235***
Secondary	5	16.67		4	13.33		
Higher secondary	10	33.33		4	13.33		
Graduate & above	12	40.00		16	53.33		
Gender							
Male	10	33.33	ND	12	40	ND	*0.592***
Female	20	66.67		18	60		
Marital status							
Married	11	36.67	ND	26	86.67	ND	*0.000***
Unmarried	19	63.33		4	13.33		
Occupation							
Profession	9	30.00	ND	16	53.33	ND	*0.49***
Skilled worker	3	10.00		4	13.33		
Unemployed	1	3.33		0	0.00		
Housewife	12	40.00		8	26.67		
Others	5	16.67		2	6.7		
Economic status							
Upper Middle	16	53.33	ND	12	40.00	ND	*0.387***
Lower Middle	12	40.00		13	43.33		
Poor	2	6.67		5	16.67		
Smoker							
Non-smoker	25	83.33	ND	23	76.67	ND	*0.181***
Ex-smoker	2	3.33		5	16.67		
Smoker	4	13.33		2	6.67		
BMI (Kg/m^2)							
Below 18.5	6	20.00	22.52 ± 3.07	1	3.33	24.0 ± 2.3	*0.068**
18.5 - 25	17	56.67		22	73.33		
25 above	7	23.33		7	23.33		

*Unpaired *t*-test was used to measure the level of significance. *P*-value <0.05 was considered as a level of significance.
**Chi square test was used to measure the level of significance. *P*-value <0.05 was considered as a level of significance.
ND = Not done.

were also pipetted into respective wells of microtitre plates. Then 500 µL of Quantia immunoglobulin activation buffer was added and incubated for 5 minutes. Absorbance (A_1) was taken. 50 µL of each of three anti-human immunoglobulins (Anti IgG, Anti IgA and Anti IgM) were added to the wells of their respective microtitre plates. The plates were incubated for 5 minutes (as specified in the kit procedure) to react the antihuman immunoglobulin with the test serum and calibrator protein. After proper mixing, absorbance (A_2) was taken at 550 nm for IgG and IgA and at 405 nm for IgM. Difference between the two absorbances ($\Delta A = A_2 - A_1$) was used for calculation. The concentration of immunoglobulins was calculated against the calibration curves of each individual immunoglobulin.

Table 2: Serum immunoglobulin concentrations of vitiligo patients (n = 30) and controls (n = 30)

Immunoglobulin (g/L)		Patients (n = 30)			Controls (n = 30)			*P*-value
	Range	N	%	Mean ± SD	N	%	Mean ± SD	
IgG	< 6	8	26.67	6.95 ± 1.31	3	10	8.1± 2.22	*0.032**
	6 – 10	22	73.33		24	80		
	> 10	0	0		3	10		
IgA	< 4	15	50	4.26 ± 2.24	11	36.67	5.95 ± 3.18	*0.042**
	4 – 6	8	26.67		7	23.33		
	> 6	7	23.33		12	40		
IgM	< 3	18	60	2.67 ± 1.19	20	66.67	2.33 ± 1.4	*0.349*
	3 – 5	12	40		9	30.00		
	> 5	0	0		1	3.33		

**P*-value <0.05 was considered as a level of significance.

Table 3: Correlation between different parameters in vitiligo patients (n = 30)

		IgG	IgA	IgM
BMI (Kg/m^2)	R	0.459	0.512	0.070
	P	0.018*	0.015*	0.735
Age	R	0.069	0.196	− 0.070
	P	0.738	0.383	0.735
Duration of disease	R	0.121	0.157	0.045
	P	0.557	0.487	0.829
No. of patches	R	0.441	− 0.012	0.195
	P	0.024*	0.957	0.339

R = Pearson correlation; P = Significance (2-tailed)
**P*-value <0.05 was considered as a level of significance.

STATISTICAL ANALYSIS

The SPSS software package (Version 11.5, SPSS Inc., Chicago, Illinois, USA) was used to analyze the data. Descriptive statistics were used for all variables. Data process on categorical scale was presented as frequency, percentage, mean, and standard deviation and was analyzed by chi-square test. Comparison of immunoglobulin levels of patients and controls were performed by cross-table variables and independent sample t-test. Correlative analysis was performed to find correlation of body mass index (BMI), age, duration of the disease and number of depigmented patches on the serum immunoglobulin concentrations. All comparisons were 2-tailed, and *P* values of <0.05 were considered significant.

RESULTS

Vitiligo was present in 10 males (33.33%) and 20 females (66.67%) as given in table 1. The duration of the disease at the time of presentation ranged from 5 months to 17 years. Koebner phenomenon was found in 10 patients (33.33%). Four patients (13.33%) had a positive family history, as shown in fig. 1. The most common site of onset was the lower and upper limbs, followed by the head and neck, trunk, beck, and mucosae. Out of all patients of case group, 14 (46.7%) had symmetrical distribution of vitiligo patches and 16 (53.3%) had asymmetrical distribution. The morphological pattern of vitiligo in the patients is shown in fig. 1. Localized or focal was the most common type (60%), followed by generalized (23.33%) and acorfacial (16.67%) types.

The serum immunoglobulins profile is given in table 2. From the table, it can be found that the IgG and IgA concentrations have been decreased significantly ($P<0.05$) than the controls; whereas the change of IgM level was not significant (increased than the control group). A significant correlation was found between BMI and IgG and IgA concentrations and between number of depigmented patches and IgG concentrations. No other factors have any significant correlation with the serum immunoglobulin concentrations (table 3).

DISCUSSIONS

A female preponderance was observed in our study. This is in agreement with Martis *et al*., 2002; Akrem *et al*., 2008 who found a predominance of females in their patients; however, Handa and Kaur (1999) observed a higher incidence in men. In a recent study from the USA,

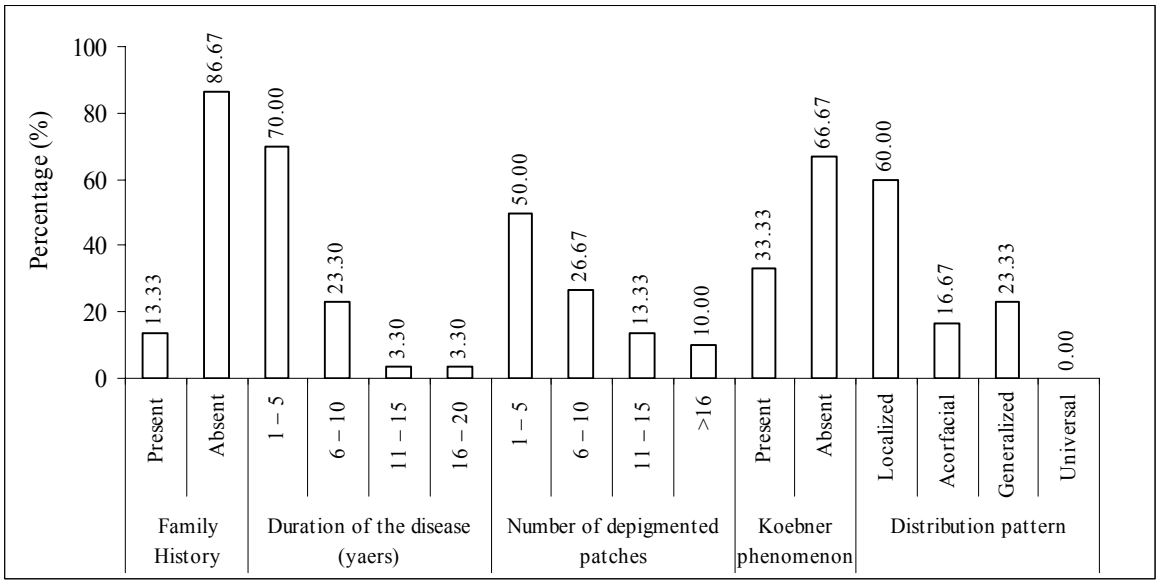

Fig. 1: Disease status of vitiligo patients (n = 30).

the frequency of vitiligo appeared to be approximately equal in males and females (Alkhateeb *et al*., 2003). The observed female preponderance can be explained by their interest in cosmetic care and frequent dermatologic consultations. A similar explanation has also been suggested by others (Kovacs, 1998; Martis *et al*., 2002).

Further, the incidence of vitiligo was 66.7% cases below 30 year of age as compared to a low incidence of 20% in individuals over 40 years of age (table 1) which means more and more younger people are getting affected with this disorder. Most of the cases (70%) were less than 5 years in duration regardless of sex, which show similarities to the other studies (Hann and Lee, 1996; Martis *et al*., 2002).

A positive family history was present in 13.3% of patients in the present study; which is higher than the findings of Hann *et al*., (13%) (1997) and Ortonne *et al*. (11.5%) (1983); but less than that of Akrem *et al*. (17.8%) (2008). The positive family history of vitiligo indicates a genetic predisposition. The presence of vitiligo in monozygous twins and the occurrence of HLA-DR4-associated vitiligo in black Americans lend support to this theory (Cho *et al*., 2000; Handa and Dogra, 2003). However the non occurrence of vitiligo in other family members throughout their life, strengthen our assumption that genetic tendency is far from significant, unless and until there is repeated insult on the melanocytes.

The development of vitiligo at the site of physical trauma is a koebner phenomenon. This may be explained as due to release of antigens of injured melanocytes into the blood and production of antibodies against them resulting in further loss of melanocytes (Ramaiah *et al*., 1989). Koebnerization was observed in a larger number of our patients (33.33%) which is a threatening condition; but others have reported this phenomenon in 18.4% (Akrem *et al*., 2008) and 5% (Handa and Kaur, 1999) of their study subjects.

In the present study, the serum IgG and IgA level was found to be significantly lower than the control subjects; that is well supported by the previous report that patients with vitiligo had a statistically significant decrease in helper cells and helper/suppressor ratios in comparison with control subjects (Grimes *et al*., 1986). These findings tend to suggest that aberrations in cell-mediated immunity may be operative in the pathogenesis of vitiligo. Moreover an insignificant increase in IgM level was also observed in vitiligo patients compared to the controls. Whether or not the change in immunoglobulin concentrations is a cause or an effect needs further research in large-scale prospective studies. Again utility of this type of immunoglobulin profiling in vitiligo patients for diagnosis could be a greater focus for researchers. These findings could direct our attention towards considering compromised immunity as a part of healthcare of this group of patients.

REFERENCES

Akrem J, Baroudi A, Aichi T, Houch F and Hamdaoui MH (2008). Profile of vitiligo in the south of Tunisia. *Int. J. Dermatol*., **47**: 670-674.

Al Badri AM, Foulis AK, Todd PM, Garioch JJ, Gudgeon JE, Stewart DG, Gracie JA and Goudie RB (1993). Abnormal expression of MHC class II and ICAM-1 by melanocytes in vitiligo. *J. Pathol.,* **169**: 203-206.

Alkhateeb A, Fain PR, Thody A, Bennett DC and Spritz RA (2003). Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their relatives. *Pigment. Cell. Res.*, **16**: 208-214.

Arican O and Kurutas EB (2008). Oxidative stress in the blood of patients with active localized vitiligo. *Acta. Dermatoven. APA.*, **17**(1): 12-16.

Arýcan Ö (2004). Vitiligoda etyoloji, patogenez ve klinik. *J. Kartal. Training. Res. Hosp.,* **5**: 55-60.

Chatain C, Ring J and Schallreuter KU (1994). Total serum immunoglobulins and atopic symptoms in patients with vitiligo. *Dermatology.,* **189**(1): 27-31.

Cho S, Kang HC and Hahm JH (2000). Characteristics of vitiligo in Korean children. *Pediatr. Dermatol.*, **17**: 189-93.

Dell'anna ML, Urbanelli S, Mastrofrancesco A, Camera E, Iacovelli P, Leone G, Manini P, D'Ischia M and Picardo M (2003). Alterations of mitochondria in peripheral blood mononuclear cells of vitiligo patients. *Pigment. Cell. Res.*, **16**: 553-559.

Farrokhi S, Farsangi-Hojjat M, Noohpisheh MK, Tahmasbi R and Rezaei N (2005). Assessment of the immune system in 55 Iranian patients with vitiligo. *J. Eur. Acad. Dermatol. Venereol.*, **19**: 706-711.

Gauthier Y, Cario AM and Taieb A (2003). A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? *Pigment. Cell. Res.*, **16**(4): 322-332.

Grimes PE, Ghoneum M, Stockton T, Payne C, Kelly AP and Alfred L (1986). T cell profiles in vitiligo. *J. Am. Acad. Dermatol.*, **14**: 196-201.

Handa S and Dogra S (2003). Epidemiology of childhood vitiligo: a study of 625 patients from North India. *Pediatr. Dermatol.*, **20**: 207-210.

Handa S and Kaur I (1999). Vitiligo: clinical findings in 1436 patients. *J. Dermatol.* **26**: 653-657.

Hann SK, Chun WH and Park YK (1997). Clinical characteristics of progressive vitiligo. *Int. J. Dermatol.*, **36**: 353-355.

Hann SK and Lee HJ (1996). Segmental vitiligo; clinical findings in 208 patients. *J. Am. Acad. Dermatol.*, **35**: 671–674.

Khanam M, Azad MAK, Ullah MA, Ahsan MS, Bari W, Islam SN, and Hasnat A (2008). Serum immunoglobulin profiles of conversion disorder patients. *German Journal of Psychiatry*, **11**(4): 141-145.

Kent G and Al Abadie MSK (1996). Psychologic effects of vitiligo: a critical incident analysis. *J. Am. Acad. Dermatol.*, **35**: 895-898.

Kovacs SO (1998). Vitiligo. *J. Am. Acad. Dermatol.*, **38**: 647-666.

Majumder PP, Nordlund JJ and Nath SK (1993). Pattern of familial aggregation of vitiligo. *Arch. Dermatol*., **129**: 994-998.

Martis J, Bhat R, Nandakishore B and Shetty JN (2002). A clinical study of vitiligo. *Indian. J. Dermatol. Venereol. Leprol.,* **68**: 92-93.

Mittal R, Popli R, Jassal J and Kumar D (1994). Immunological study in vitiligo and contact depigmentation. *Indian. J. Dermatol. Venereol. Leprol.*, **60**(2): 92-94.

Naughton GK, Reggiardo MD and Bystryn J-C (1986). Correlation between vitiligo antibodies and extent of depigmentation in vitiligo. *J. Am. Acad. Dermatol.*, **15**: 978-981.

Ogg GS, Dunbar PR, Romero P, Chen JL and Cerundolo V (1998). High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. *J. Exp. Med.*, **188**: 1203-1208.

Ortonne JP, Moscher DB and Fitzpatrick TB (1983). Vitiligo and other hypomelanoses of hair and skin. In: Ortonne JP, Mosher DB, Fitzpatrick TB, eds. *Topics in Dermatology*. Plenum Medical Book Co., New York, pp.257-258.

Perfetti L, Cespa M, Nume A and Orecchia G (1991). Prevalence of atopy in vitiligo. A preliminary report. *Dermatologica.*, **182**(4): 218-220.

Ramaiah A, Puri N and Mojamdar M (1989). Etiology of vitilgo. A new hypothesis. *Acta. Dermatol. Venereol.*, **69**: 323-326.

Taeib A (2000). Intrinsic and extrinsic pathomechanisms in vitiligo. *Pigment. Cell. Res.,* **13**(Suppl. 8): 41-47.

Van den Wijngaard R, Wankowicz-Kalinska A, Le Poole C, Tigges B, Westerhof W and Das P (2000). Local immune response in skin of generalised vitiligo patients. Destruction of melanocytes is associated with the prominent presence of CLA+ T cells at the perilesional site. *Lab. Invest.,* **80**: 1299-1309.