CHAPTER VII

ROLE OF ACETYLCHOLINE ESTERASE IN THE PATHOGENESIS OF VITILIGO

7.1. Introduction

Vitiligo is a depigmenting disorder resulting from the loss of melanocytes in the skin and affects 1-2% of the world population (Moscher et al 1999; Nordlund and Ortonne 1998). The incidence of vitiligo is found to be 0.5-2.5% in India (Handa and Kaur 1999; Das et al 1985). Gujarat and Rajasthan states have the highest prevalence i.e. ~8.8% (Valia and Dutta 1996). Vitiligo is classified into non-segmental and segmental clinical types. Non-segmental type includes vulgaris, acrofacial, focal and universal sub-types. In vulgaris the lesions are found in typical zones with symmetrical distribution. Acrofacial sub-type of non-segmental vitiligo affects face and distal extremities. In focal vitiligo one or more patches are found in one area but not in segmental pattern. In universal vitiligo the depigmentation involves more than 80% of the body (Hann and Nordlund 2000). In segmental vitiligo one or more macules are found in dermatomal unilateral distribution.

Though vitiligo is extensively addressed in the past five decades, its etiology is still being debated (Taieb 2000; Le Poole et al 1993; Ortonne and Bose 1993; Agrawal et al 2001; Cucchi et al 2003; Ongenae et al 2003; Boisseau-Garsuad et al 2002). Several hypotheses were proposed about the pathogenesis of vitiligo and neurochemical hypothesis considers a cutaneous defect of the melanocytic system (Taieb et al 2000). According to Taieb (2000) the origin of segmental and non-segmental vitiligo may be different. Local/systemic factors affect the homeostasis of the epidermal melanin unit in segmental vitiligo, which is restricted to a limited cutaneous territory whereas an impaired redox status of the epidermal melanin unit acts as the primary defect further leading to inappropriate immune response in non-segmental vitiligo. The neural theory is more related with segmental vitiligo whereas the autoimmune theory is implicated in non-segmental vitiligo (Taieb 2000). There are no reports on systemic acetylcholine esterase (AChE) levels in vitiligo patients. Hence we made an attempt to explore

whether there is any involvement of systemic AChE in precipitating vitiligo in Gujarat patients. In this study we show analysis of blood AChE activity in different age groups of vitiligo patients compared to controls; segmental and non-segmental types; active and stable forms of vitiligo.

7.2. Materials and methods

For the estimation of blood AChE activity, one twenty one vitiligo patients were divided into different age groups (5-15 yrs, 16-25 yrs, 26-35 yrs 36-45, 46-55 yrs) and clinical types after written informed consent had been obtained. The patients had no other associated diseases. One twenty six controls were age matched healthy consenting volunteers. Blood AChE activity was assayed according to the method of Reiner et al (2000).

Estimation of Acetylcholine esterase activity

Principle:

Acetylcholine esterase catalyzes the hydrolysis of acetylcholine to thiocholine. The rate of production of thiocholine is measured by following the reaction of thiocholine with DTNB, which produces a yellow colored anion due to the formation of 5-thio-2-nitrobenzoic acid. The rate of formation of the yellow anion is measured at 412 nm. The activity of AChE was expressed as micromoles of acetylthiocholine (ATCh) produced per gram hemoglobin per minute.

$$\text{Acetyl thiocholine iodide} \xrightarrow{\text{AChE}} \text{Thiocholine} + \text{Acetate}$$

$$\text{Thiocholine} + \text{DTNB} \longrightarrow \text{5 Thio 2 nitrobenzoic acid} + \text{Oxidized thiocholine}$$

Reagents	
Phosphate buffer (pH 7.0)	0.1 M
Acetylthiocholine iodide	10 mM
DTNB	1 mM, dissolved in phosphate buffer
Ethopropazine	6 mM, dissolved in absolute alcohol

Sample preparation: Hemolysate was prepared as described in the chapter 3. The hemoglobin estimation of the lysate was done by Drabkin's method as described in the chapter 3. The lysate was diluted 600 times and this diluted lysate was used as sample.

Protocol

Reagents	Test	Blank
Hemolysate	1.7 ml	1.7 ml
DTNB	0.99 ml	0.99 ml
Ethopropazine	0.01 ml	0.01 ml
Distilled water	---	0.3 ml
Incubated for 10 minutes		
ATChI	0.3 ml	---

Calculation

$$\frac{\Delta \text{ OD}}{13600} \times \frac{3}{1.7} \times \frac{\text{Dilution factor (600)}}{\text{Hb Conc of the lysate}}$$

13600 is the molar extinction coefficient of DTNB

Unit: µmoles of ATChI hydrolyzed /minute/gHb

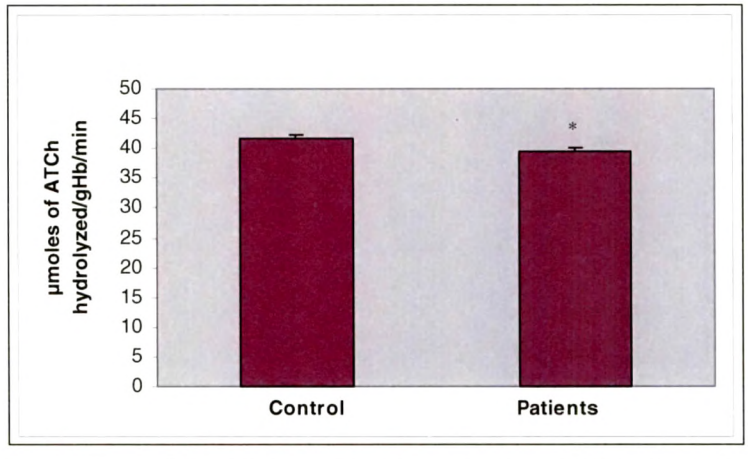
Statistical analysis

Results in vitiligo patients and controls were compared using the paired students' t test. One way analysis of variance (ANOVA) was used to determine significant differences in AChE enzyme activities between different age groups and different clinical types of vitiligo utilizing statistical software program Prism and $p \leq 0.05$ was considered significant.

7.3. Results

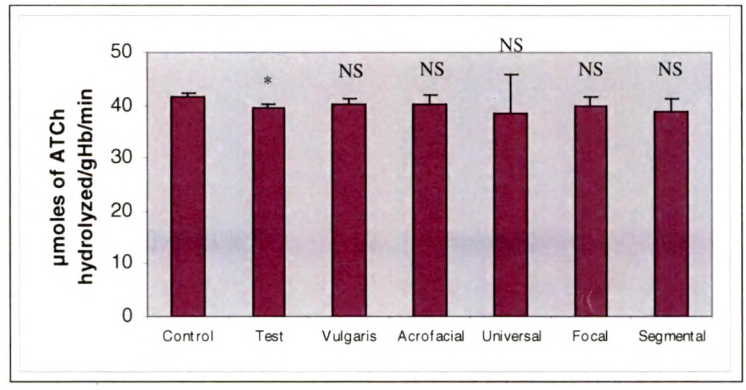
The present study is an attempt to analyze blood AChE activity in vitiligo patients compared to controls. Acetylcholine esterase showed significant decrease in vitiligo patients compared to controls (Figure 1). Patients were also classified into different clinical types for our further analysis. However there is no significant difference in the AChE activity in different clinical types (Figure 2). Further, AChE activity in vitiligo patients was analyzed in different age groups to find whether it plays any role in the pathogenesis of vitiligo in different age groups (Figure 3). The age group 16-25 showed a significant decrease in AChE activity compared to controls. Other groups have not shown any difference in the activity compared to controls (Figure 3).

Figure 1. Acetylcholine esterase activity in the in erythrocytes of control and vitiligo patients#



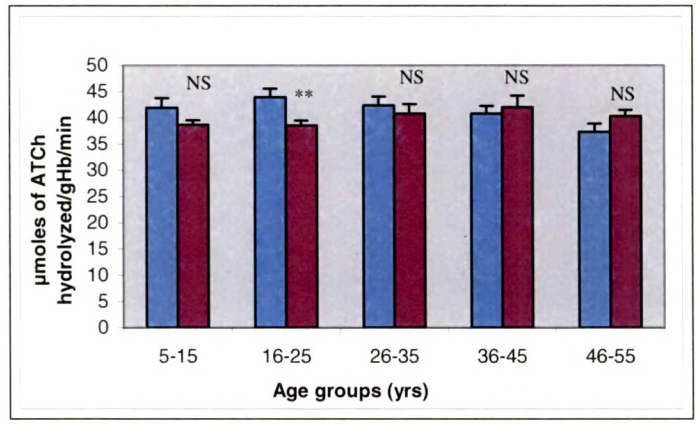
Values are given as mean ± SE of 126 observations in controls and 121 individual observations in patients. *, $p<0.05$.

Figure 2. Acetylcholine esterase activity in the erythrocytes of controls and different clinical types of vitiligo#



Values are given as mean ± SE of 126 individual observations in controls; 71 individual observations vitiligo vulgaris; 20 individual observations in focal vitiligo; 19 individual observations acrofacial vitiligo; 10 individual observations in segmental vitiligo and 4 observations in universal vitiligo respectively. *, $p<0.05$; NS, Non significant

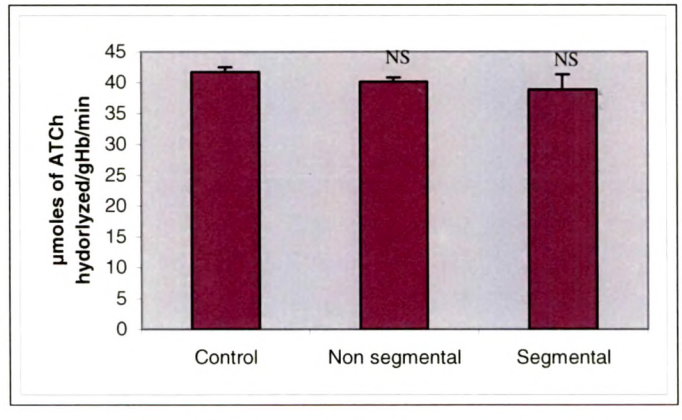
Figure 3. Acetylcholine esterase activity in the in erythrocytes of control and different age groups of vitiligo patients#



Values are given as mean ± SE of 19 and 22 individual observations in controls and patients in 5-15 age group; 45 and 57 individual observations in controls and patients in 16-25 age group; 24 and 18 individual observations in controls and patients 26-35 age group and 25 and 15 individual observations in controls and patients in 36-45 age group; 13 and 12 individual observations in controls and patients in 46-55 age group respectively. Blue bars are of controls and pink bars are of vitiligo patients. **,$p<0.01$; NS, non significant

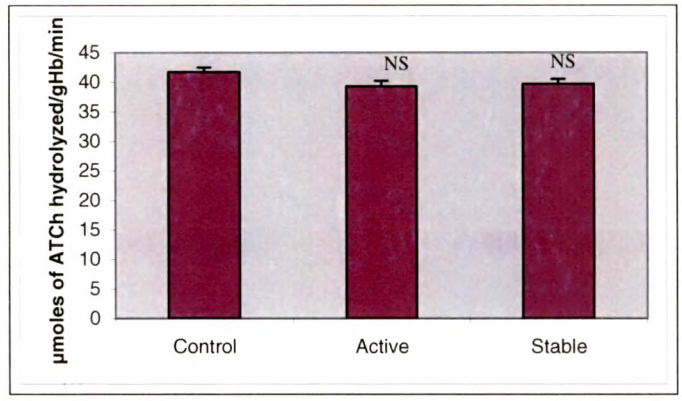
Segmental and non-segmental types also did not show any significant difference in the AChE activity (Figure 4). Also we analyzed active and stable vitiligo, but we did not find any significant difference (Figure 5). In order to study the role of AChE enzyme in the genetic predisposition of vitiligo, we have analyzed its activity in patients with positive family history as well as negative family history. However no significant difference was observed in AChE activity (Figure 6). Further our analysis by ANOVA in different age groups and different clinical types showed no significant difference of the AChE activity among the groups.

Figure 4. Acetylcholine esterase activity in the erythrocytes of controls, segmental and non-segmental vitiligo#



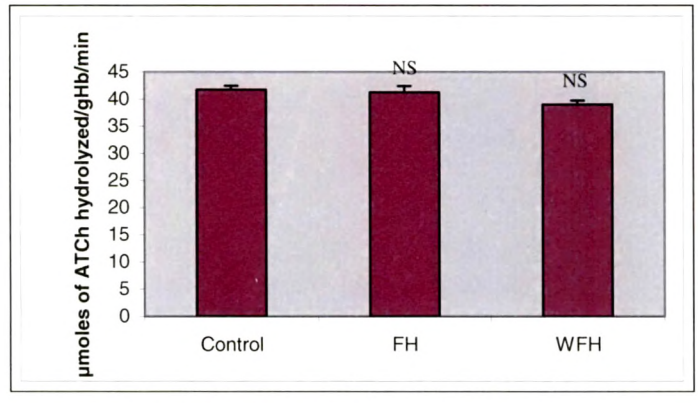
Values are given as mean ± SE of 126 individual observations in controls; 111 individual observations in non-segmental vitiligo and 10 individual observations in segmental vitiligo respectively. NS, non significant

Figure 5. Acetylcholine esterase activity in the erythrocytes of controls, active and stable vitiligo#



Values are given as mean ± SE of 126 individual observations in controls; 60 individual observations in active vitiligo and 61 individual observations in stable vitiligo respectively. NS, non significant

Figure 6. Acetylcholine esterase activity in the erythrocytes of controls, patients with positive family history and patients with negative family history of vitiligo#



Values are given as mean ± SE of 126 individual observations in controls; 29 individual observations in patients with positive family history and 92 individual observations in patients with negative family history respectively. NS, non significant, FH, With family history, WFH, Without family history

7.4. Discussion

Melanocytes are neural crest derived cells with an embryological link to the nervous system (Reedy et al 1998). According to neural hypothesis neurochemical mediators including acetylcholine secreted by the nerve endings are toxic to melanocytes, which leads to their destruction in vitiligo patients. Thus segmental vitiligo may be associated with the dysfunction of cholinergic sympathetic nerves (Taieb 2000; Koga 1997). Acetylcholine esterase activity is found to be lowered in vitiliginous skin during depigmentation (Iyengar 1989), suggesting that acetylcholine may aggravate the process of depigmentation in vitiligo (Iyengar 1989). There are a few reports on the AChE activity in vitiligo. Lower AChE levels are reported in the vitiliginous skin during depigmentation process (Iyengar 1989). Further, a possible cholinergic involvement in vitiligo has been reinforced by demonstrating decreased sweating in the depigmented epidermis of these patients (Elwary et al 1997). Schallreuter et al (2004) studied H_2O_2 regulation of AChE and showed H_2O_2 mediated oxidation of AChE, thus emphasizing the role of oxidative stress in precipitating vitiligo (Schallreuter et al 2004). These results in conjunction with our studies on oxidative stress hypothesis (Agrawal et al 2004; Agrawal 2002; Shajil and Begum 2006) provide evidence that AChE may be inactivated at higher concentrations of H_2O_2. Inactivation of the enzyme is due to the oxidation of Trp^{432}, Trp^{435} and Met^{436} residues by H_2O_2 (Schallreuter et al 2004). Our study shows a significant decrease in the blood AChE activity in vitiligo patients compared to controls (Figure 1) along with increased levels of lipid peroxidation levels (Agrawal et al 2004; Agrawal 2002), which is an indicator of oxidative stress. Thus accumulated acetylcholine may lead to the destruction of melanocytes in vitiligo. It has also been shown that acetylcholine has an inhibitory effect on DOPA oxidase activity in the melanocytes and inhibits the pigment production (Iyengar 1989). However, we could not find any significant change in the AChE activity in segmental vitiligo (Figure 4), which is attributed to be of

neurochemical origin. This is the first report showing that AChE may be playing a role in the pathogenesis of Gujarat vitiliginous patients (Shajil et al 2006).

7.5. References

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