

CHAPTER V
SUPEROXIDE DISMUTASE 1 PROTEIN CONTENT IN
RELATION TO SOD ACTIVITY IN VITILIGO PATIENTS
COMPARED TO CONTROLS

5.1. Introduction

Vitiligo is an idiopathic, acquired, circumscribed hypomelanotic skin disorder, characterized by milky white patches of different size and shape. 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; Cucchi et al 2003; Ongena et al 2003; Boisseau-Garsuad et al 2002). Several hypotheses were proposed to explain the pathogenesis of vitiligo and oxidative stress hypothesis considers a systemic involvement in the course of the disease (Picardo et al 1994; Yildirim et al 2003). Oxidative stress could act as the initial triggering event in melanocyte degeneration.

Superoxide dismutases are metalloenzymes found widely distributed in prokaryotic and eukaryotic cells (Fridovich 1995). Three distinct isoforms of SOD have been identified in mammals. Two isoforms of SOD have Cu and Zn at their catalytic center and are localized to either intracellular cytoplasmic compartments (CuZn or SOD1) or to extracellular spaces (EC SOD or SOD3). SOD2 is localized to mitochondria and has Manganese (Mn) as the prosthetic group. SOD1 has a molecular mass of about 32,000 Da and has been found in the cytoplasm, nuclear components and lysosomes of mammalian cells (Chang et al 1988; Keller et al 1991). The SOD1 gene is localized on chromosome 21(21q22) in humans.

Our biochemical studies showed that the activity of SOD1 is significantly higher in vitiligo patients compared to controls (Agrawal et al 2004; Shajil and Begum 2006). The aim of this study was to find whether the higher SOD1 activity obtained in vitiligo patients is due to the increased amount of SOD1 protein content or the presence of any mutation in the *SOD1* gene, which could enhance the SOD1 activity.

5.2. Materials and methods

Blood samples were collected from 124 vitiligo patients without any other associated diseases and also from 126 age-matched healthy consenting

volunteers for the estimation of antioxidant parameters. Out of 124 vitiligo patients, fifty patients who showed high SOD1 activity and fifty controls were selected for the western blot analysis. The hemolysate containing 0.5 g% Hb was run on 15% SDS PAGE under reducing conditions (160 mM DTT) and electro blotted onto PVDF membrane. The membranes were blocked with 1% BSA in PBS for two hours, added anti superoxide dismutase 1 (Cu/Zn) sheep polyclonal Ab (Calbiochem, Germany) diluted to 1: 5000 with PBS containing 0.1% Tween 20 and incubated for two hours at room temperature. Washed three times with PBS containing 0.2% Tween 20 and added 1:5000 diluted (PBS containing 0.1% Tween 20) secondary antibody (Anti sheep IgG, Sigma, USA) and incubated for 1 hour at room temperature. Washed three times with PBS containing 0.15% Tween 20 and developed with DAB (Diaminobenzidine) in PBS containing NiCl₂ and H₂O₂.

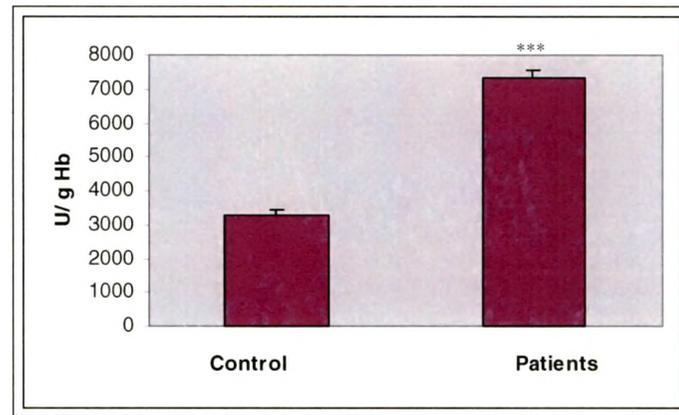
Statistical analysis

Results on vitiligo patients and controls were compared using the paired student's *t* test utilizing statistical software program Prism and $p \leq 0.05$ was considered significant.

5.3. Results

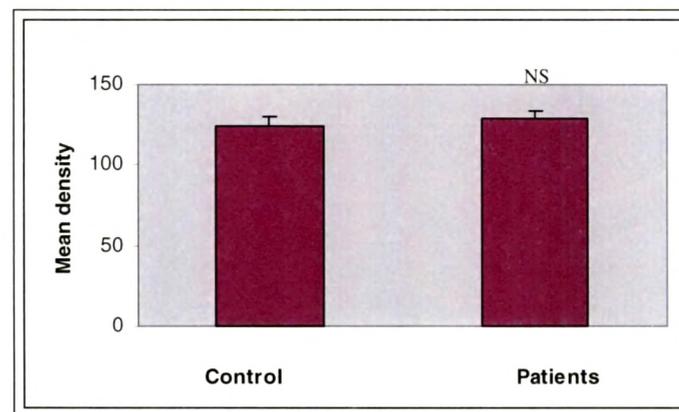
The present study is an attempt to analyze blood SOD1 activity and its protein levels in vitiligo patients compared to controls. SOD1 activity showed significant increase in vitiligo patients compared to controls ($p < 0.0001$) (Figure 1). However the western blot and densitometric analysis showed that there is no significant change in SOD1 protein levels in vitiligo patients compared to controls (Figure 2 & Figure 3).

Figure 1. Superoxide dismutase 1 activity in erythrocytes of controls and vitiligo patients#



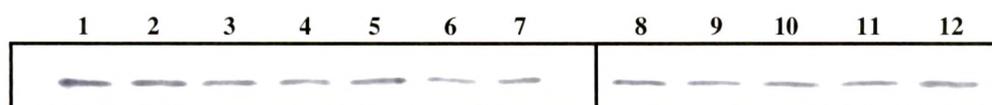
Values are given as mean \pm SE of 50 individual observations in controls and patients. ***, $p < 0.001$

Figure 2. Densitometric analysis of SOD 1 protein levels in vitiligo patients compared to controls#



Values are given as mean \pm SE of 50 individual observations in controls and patients. NS, non significant.

Figure 3. Western blot analysis of erythrocyte SOD 1 protein levels in vitiligo patients and controls



Patients: 1, 2, 3, 4, 8, and 9; Control: 5, 6, 7, 10, 11, and 12

5.4. Discussion

Significant increase in the SOD1 activity was observed in vitiligo patients compared to controls ($p < 0.0001$) (Figure 1). This is in accordance with Chakraborty et al (1996). However Picardo et al (1994) reported that SOD1 activity in erythrocytes of vitiligo patients was not significantly different from healthy age matched controls. The aim of this study was to verify whether the increase in the SOD1 activity in vitiligo patients is due to increased protein levels or presence of any mutation/s that may enhance the activity of SOD1. Western blot analysis and corresponding densitometric analysis showed that the protein levels of SOD1 remained same in both controls and patients (Figure 2 & Figure 3). The above results show that the increased activity in vitiligo patients is not due to the increase in the SOD1 protein levels (Figure 2 & Figure 3). Thus our results suggest that mutation/s in the exonic regions of SOD1 gene may enhance the activity of this enzyme. There are no reports available which showed an increased activity of the enzyme without a change in the protein content in different groups i.e. controls and patients. However, Sandstrom et al (1994) showed that a 10 fold increase in human plasma EC SOD (SOD3) content was caused by a mutation in heparin binding region (Arg213Gly). The high plasma activity is because of the accelerated release of SOD3 from the tissue interstitium heparan sulphate to the vasculature and accompanied by significant reduction in tissue EC SOD activities (Sandstrom et al 1994). This is the first study showing the enhanced SOD1 activity in vitiligo patients compared to control population without any change in the protein content.

5.5. References

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