

**OXIDATIVE STRESS IN THE PATHOGENESIS OF VITILIGO****DR.D. MADHAVI^{1*}, DR.T.E.DIVYAMALINI² AND DR.C.V.SARADA³***1MD Biochemistry, Osmania Medical College , Hyderabad;Consultant Biochemist, Yashoda Hospitals, Secunderabad India**2 MD Biochemistry, Osmania Medical College , Hyderabad, India**3 MD Biochemistry, S.V Medical College, Tirupathi; Professor in Biochemistry , Gandhi Medical college , Hyderabad, India***ABSTRACT**

Vitiligo - specific, common, often heritable, acquired disorder characterized by circumscribed milky white cutaneous macules.. The disease shows no racial/gender predilection and can occur at any age. The incidence is found to be 1-2%. Among the various theories available for explaining the pathogenesis of vitiligo, this study evaluates the role of oxidative stress in the disease pathogenesis. In this study 100 cases of vitiligo patients were evaluated in two age groups as follows Children (8-17 years) Young adults(18-30years) Serum levels of malondialdehyde, catalase, zinc were estimated in these patients. The values of these parameters were compared with 100 healthy controls who were grouped in the same way as cases on the basis of age. There was a statistically significant increase in serum malondialdehyde and decrease in serum catalase and serum zinc in the both groups of cases as compared to their age based controls. Thus it can be concluded that oxidative stress is the primary and also the major factor contributing to the pathogenesis of vitiligo. Thus the use of antioxidant therapy in vitiligo patients could yield a better outcome in this psychologically disturbing depigmenting disorder

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INTRODUCTION

Vitiligo is a specific, common, often heritable, acquired disorder characterized by well – circumscribed depigmented cutaneous patches, is restricted to a limited cutaneous territory (focal/segmental vitiligo) or generalized in symmetric patches, devoid of identifiable melanocytes. Incidence of the disease is between 1 and 2 percent. All races are affected, both sexes equally and the disease may develop at any age. This disease, though not fatal, causes serious psychological impact on the patient affecting the lifestyle of the patient. Its confusion with leprosy causes great social stigma to the patient. The pathogenesis of vitiligo is still unclear which is evident by the number of hypotheses proposed. The aim of this case-control study done in 100 cases is to evaluate the role of oxidative stress in the pathogenesis of vitiligo. This is done by estimating the levels of serum malondialdehyde as an indicator of oxidative stress, serum catalase and serum zinc indicative of antioxidant capacity.

MATERIALS AND METHODS

The parameters were analyzed in 100 patients of newly diagnosed vitiligo, who were divided into 2 groups children (8-17 years) and young adults(18-30years). The results were compared with 100 age and gender matched healthy controls without any dermatological disorders. The study thus consisted of four groups –

- CCA CHILDREN CASES (50)
- CC CHILDREN CONTROLS (50)
- ACA ADULT CASES (50)
- AC ADULT CONTROLS (50)

INCLUSION CRITERIA

- Newly diagnosed cases of vitiligo
- Age group of 8-30 years

EXCLUSION CRITERIA

- Patients who are already undergoing treatment for vitiligo
- Patients who are on any form of zinc supplementation.
- Patients who are suffering from any other dermatological disorders.

INVESTIGATIONS

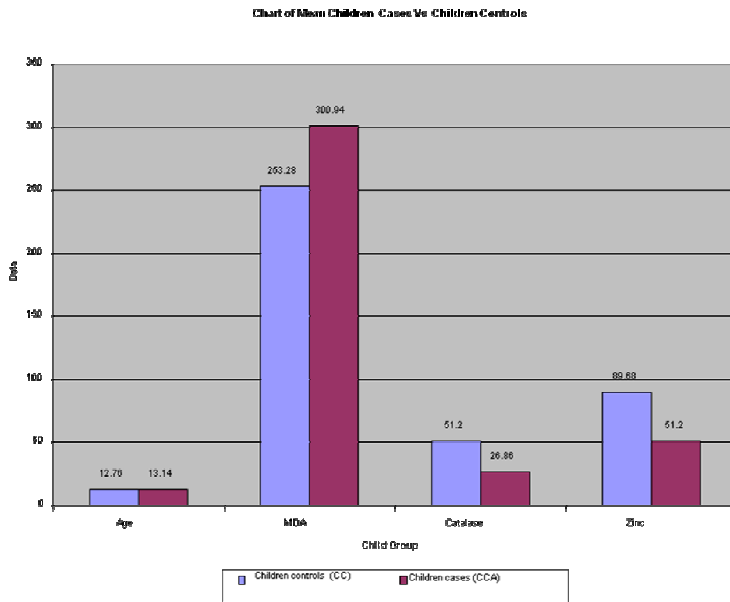
- Serum malondialdehyde was done by Thiobarbituric acid reactive substance assay (TBARS)¹The malondialdehyde in the serum reacts with thiobarbituric acid in an acidic medium and gives rise to color complex (pink), which is measured at 520nm against distilled water in spectrophotometer.
- Serum catalase A spectrophotometric assay for serum catalase activity was developed. It was a combination of optimized conditions and the spectrophotometric assay of hydrogen peroxide based on the formation of its stable complex with ammonium molybdate²
- Serum zinc
Estimation of serum zinc was done by NITROPAPS method (colorimetric)³ Zinc in alkaline medium reacts with Nitro-PAPS(2-5-Nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamine)phenol) to form a purple coloured complex. Intensity of the complex formed is directly proportional to the amount of zinc present in the sample

RESULTS

The results were represented in the form of tables and bar diagrams. The data has been statistically analysed by using mean, standard deviation. Independent sample 't' test and 'p' value were used to assess the significance of difference of means between the cases and control groups. Pearson correlation was used to assess the correlation between different parameters in the groups analyzed and correlation coefficient was computed. Specificity and sensitivity of the different parameters in analyzing the status of oxidative stress were computed by using ROC curves with the "graph pad prism" software. In the present study, compared to the respective age and gender matched controls, there was a statistically significant increase in serum MDA levels in both children(CCA)[p' value=0.001] as well as adult cases(ACA) [p' value=0.001], decrease in the catalase enzyme activity in both children(CCA) [p' value=0.001] as well as adult(ACA) groups [p' value=0.001], decrease in the serum zinc in both

children(CCA) [‘p’ value=0.001] as well as adult(ACA) groups [‘p’ value=0.001]

Bar diagrams of means in the children group



Bar diagrams showing means of adult groups

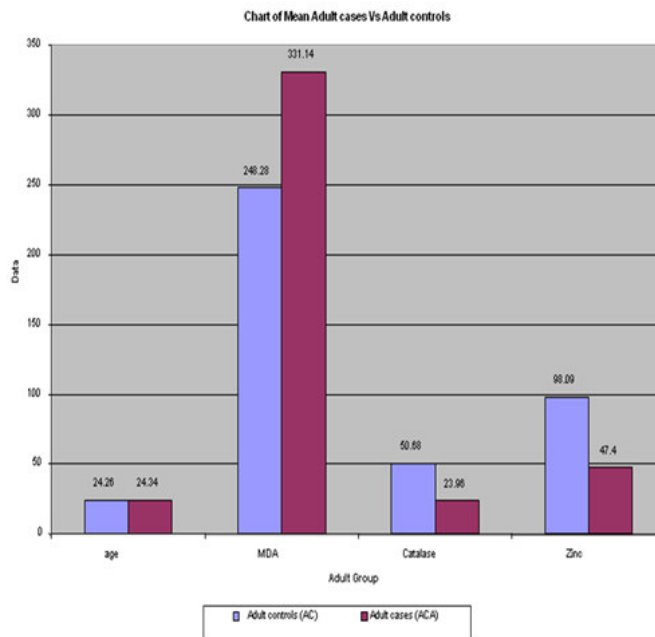


Table 1
Representing t and p values for Serum MDA.

Groups	t – value	p – Value
Children(Group CCA vs. CC)	14.11	0.001 S
Adults(Group ACA vs. AC)	19.1	0.001 S

Table 2
Representing t and p values for Serum Catalase.

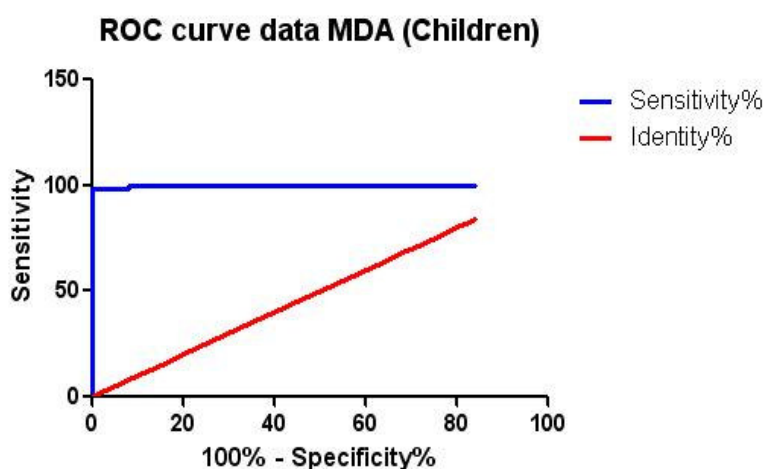
Groups	t – value	p – Value
Children(Group CCA vs. CC)	28.73	0.001 S
Adults(Group ACA vs. AC)	37.55	0.001 S

Table 3
Representing t and p values for Serum Zinc

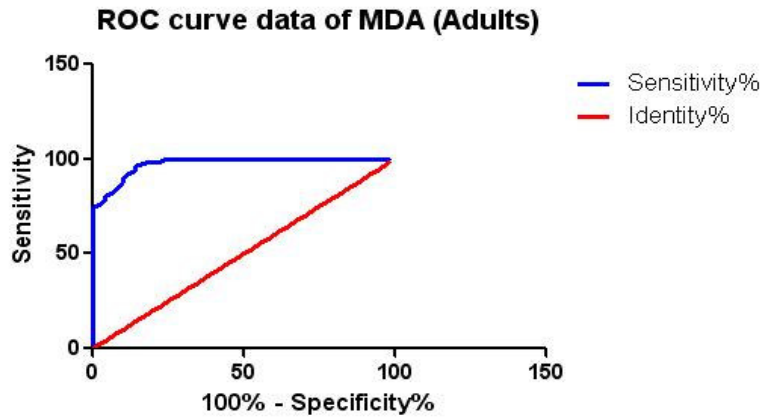
Groups	t – value	p – Value
Children(Group CCA vs. CC)	22.14	0.001 S
Adults(Group ACA vs. AC)	19.74	0.001 S

In order to assess discriminatory capacity of various parameters in differentiating different study groups the sensitivities and specifications were calculated using best cut off values. The best cut off values was calculated by plotting ROC curves by using Graph Pad Prism Software. The diagnostic accuracy is represented by area under curve(AUC)

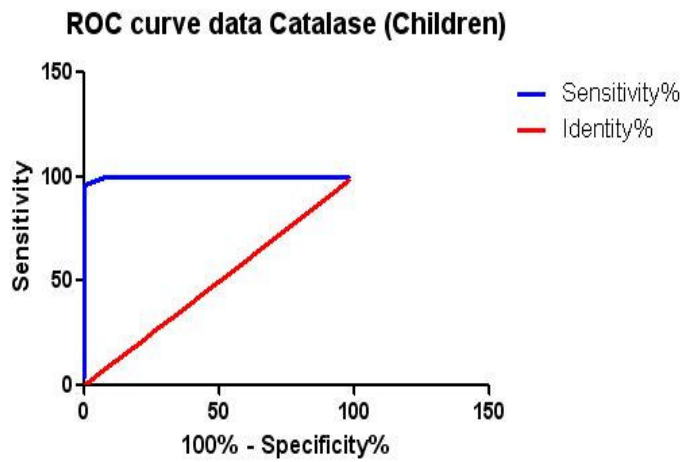
ROC curve of MDA (children)



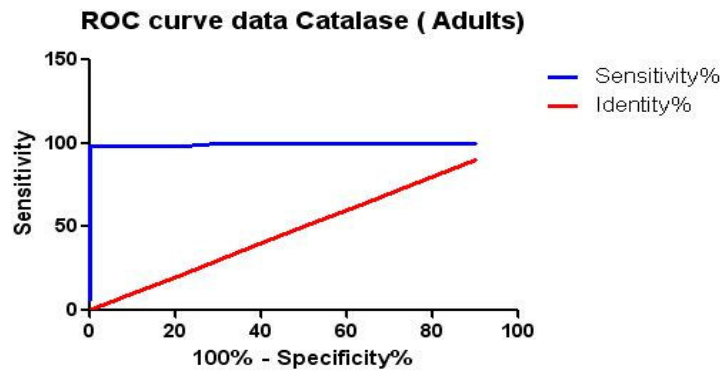
ROC curve of MDA (adults)



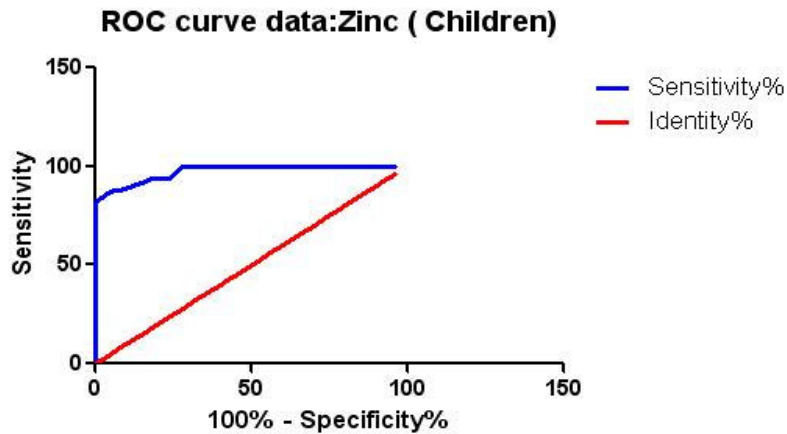
ROC curve of catalase (children)



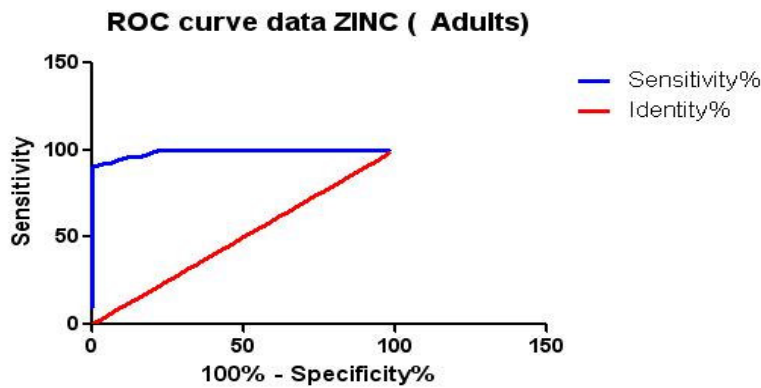
ROC curve of catalase (adults)



ROC curve of Zinc (children)

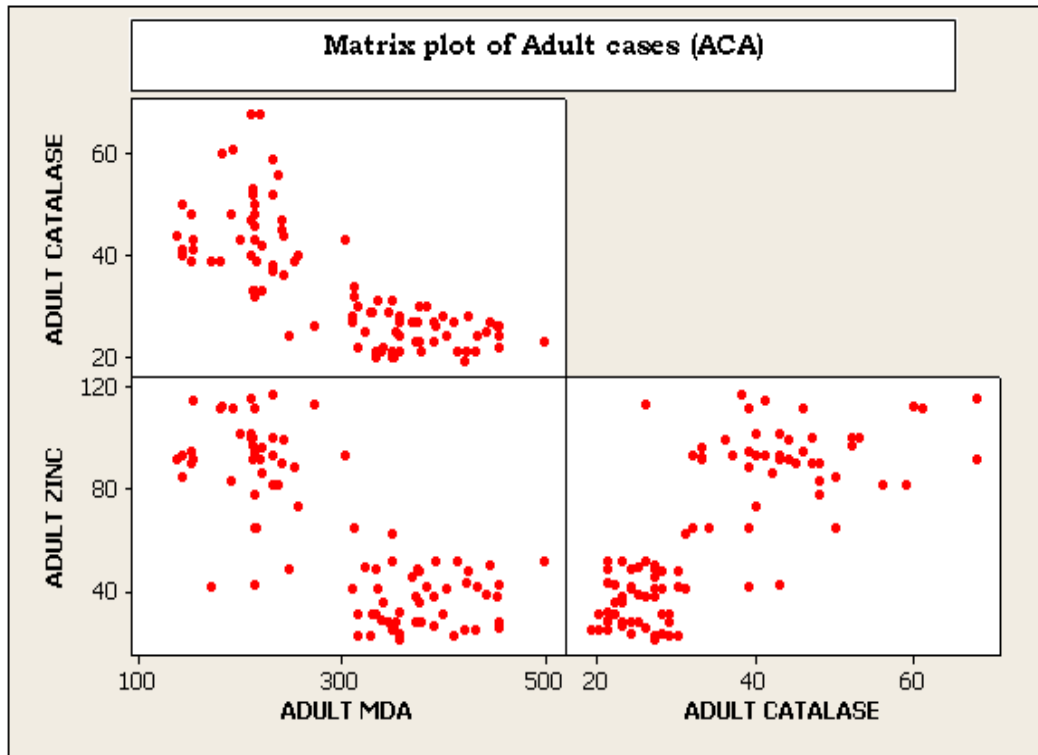


ROC curve of Zinc (adults)

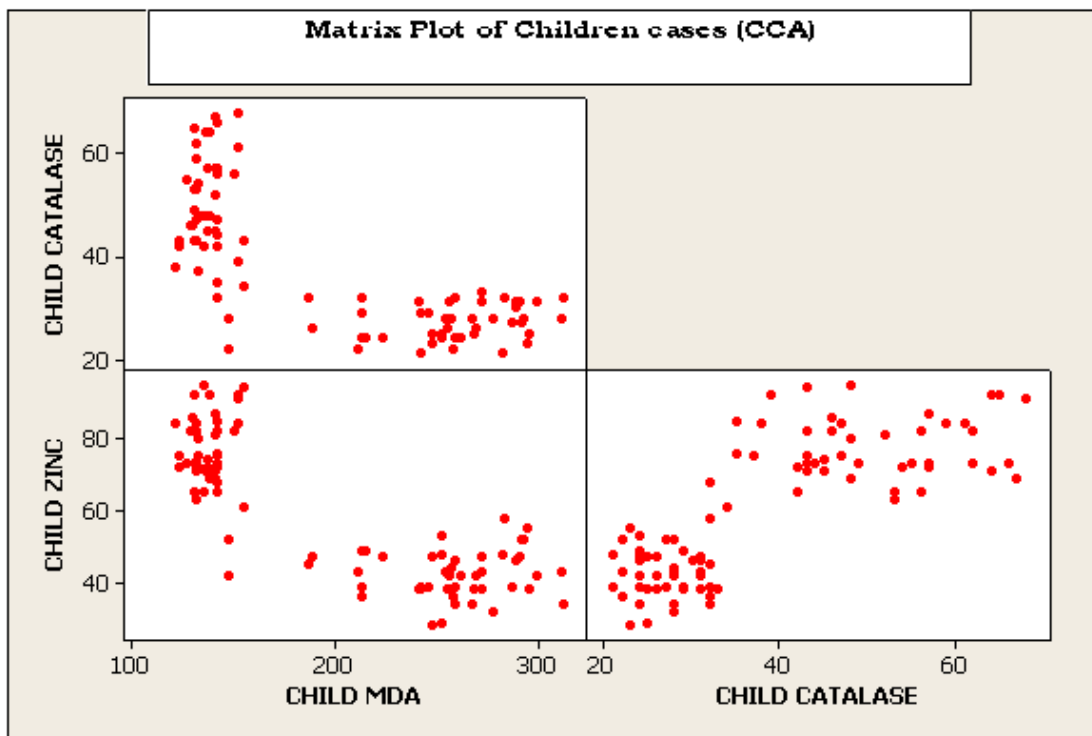


In children the most sensitive marker was serum MDA (98%) and the specificity of all the three markers was equally good approaching to 100%. In adults the most sensitive marker was serum MDA(100%) and the most specific markers were catalase and zinc.(100%) A positive correlation was seen between the parameters catalase and zinc [$r=0.57$] in the children age group. There was no significant correlation seen between the parameters in the adult age group.

Matrix plot of adult cases(ACA)



Matrix plot of children cases(CCA)



DISCUSSION

Vitiligo is an acquired idiopathic, cutaneous disease characterized by depigmented patches. The exact pathogenesis of this disease has not been elucidated yet. Oxidative stress is induced by the over production of reactive oxygen species (ROS) which oxidize lipids, proteins and DNA and cause cellular damage and eventually cell death and play a role in the pathogenesis of several diseases. Atherosclerosis, stroke, heart diseases, diabetic vascular disease, cancer and even early ageing are thought to occur as a result of oxidative damage. Oxidative stress was implicated in the pathogenesis of vitiligo^{4,5}. A dysregulation of melanogenesis leads to the formation of the unstable 4- α hydroxy BH₄ intermediate which leads to the production of quinonoid BH₂ and H₂O₂. H₂O₂ has been shown to oxidize BH₄ to 6-biopterin which is toxic to melanocytes in vitro.⁶ There is increased production of free radicals via H₂O₂ generation. This results in oxidative stress which leads to oxidative damage, resulting in cellular injury.⁷ Increased MDA levels are the end product of lipid peroxidation. A statistically significant increase in serum MDA levels observed in the present study is in concordance with earlier studies^{8,9,10}. It had been proposed that mutations in/near the CAT gene may

contribute to the decrease in activity of catalase enzyme. This leads to the accumulation of hydrogen peroxide, further providing evidence for the 'oxidative stress hypothesis of vitiligo'.¹¹ High concentrations of H₂O₂ can lead to deactivation of catalase.¹² A statistically significant decrease in serum catalase levels observed in the present study is in concordance with earlier studies^{13,14,15,16,17,18}. The antioxidant property of zinc is thought to be because of its association with the antioxidant enzyme superoxide dismutase. Zinc may induce the synthesis of metallothionein, sulfhydryl rich proteins that protect against injury from free radicals.⁴ It acts by replacing redox active molecules such as copper and iron at critical sites in cell membranes and proteins. The decrease in serum zinc levels observed in the present study is also supported by the other studies^{19,20}. The above findings are in concordance with the studies of] ²¹who also showed an imbalance between oxidant and antioxidant status in vitiligo patients. As observed in the present study and also supported by the other above stated studies, oxidative stress is the primary^{15,22} and also the major factor contributing to the pathogenesis of vitiligo. Thus the use of antioxidant therapy in vitiligo patient could yield a better outcome in this psychologically disturbing depigmenting disorder.

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