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Study of oxidative stress in vitiligo

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Abstract

Vitiligo vulgaris is an autoimmune disease of the skin characterized by depigmented macules. Apart from autoimmune etiology, damage to melanocytes by oxidative stress has been postulated as one of the causative factors in pathogenesis of vitiligo. The current study was conducted at our tertiary care center to estimate oxidative stress in the blood of patients suffering from vitiligo. A total of 40 patients were included after informed consent. All treatment-naïve patients of vitiligo of >15 years of age of either sex and those patients who have not received systemic treatment in the last 3months for vitiligo or topical treatment in the last 2 weeks were included. Detailed history was taken and clinical assessment of vitiligo was done. Venous blood samples were collected for estimation of Malondialdehyde (MDA), superoxide dismutase (SOD) and Glutathione Peroxidase (GPx) which is considered as markers for oxidative stress in the blood. Majority of vitiligo cases belonged to younger age groups. A total of 60% cases were in the age group of 16–25years followed by 20% in 26–35years. A total of 50% cases were having active or unstable disease while 50% cases were having stable disease. As assessed by MDA, 35/36(97.22%) was found to have raised oxidant stress. As assessed by GPX, all i.e. 36/36 patients were (100%) were found to have raised oxidant stress. As assessed by SOD, 34/36 (94.44%) were found to have raised oxidant stress. Mean values of MDA in our patients were 4.3±2.75SD nmol/ml. Mean values of GPx in our patients were 2864±1008SD u/l. Mean values of SOD were 97.11±91.46SD u/ml. Mean values of MDA, GPX and SOD in matched controls were 0.91±0.21SD nmol/ml, 6058.60±1694SD u/l and 189.43±23.57SD u/ml respectively. As compared to controls, increased MDA levels and reduced GPX and SOD levels were suggestive of oxidative stress in patients of vitiligo in our study. More studies of relatively larger sample size are required to further confirm these variations of MDA, SOD and GPx in the blood.

Keywords: Vitiligo, Oxidative stress, Malondialdehyde, Superoxide dismutase, Glutathione Peroxidase.

Introduction

Vitiligo or leukoderma is an acquired autoimmune skin disorder of pigmentation characterized by well demarcated depigmented macules and patches of different sizes and shapes. Destruction of melanocytes due to autoimmune inflammatory process is considered to be the main pathogenesis of resultant depigmentation over the skin. However exact cause is unknown. Different hypothesis has been put forward with regard to pathogenesis of Vitiligo. Damage to melanocytes due to oxidative stress has been reported as one of the etiological factors in causation of viltiligo.[1-2]

Oxidative stress is postulated to be one of the etiologic factors for decreased pigmentation in the vitiliginous area through modification of melanocyte antigens. Various researchers have reported either increased antioxidant levels, no change or even decreased levels of markers like Superoxide Dismutase(SOD), Glutathione peroxidase (GPx), Malondialdehyde (MDA), Nitric Oxide (NO), and Catalase. During oxidative stress, molecular oxygen (O_2) is reduced to form superoxide radicals. Further, superoxide radicals undergo dismutase reaction to hydrogen peroxide(H2O2). This happens spontaneously or by enzyme superoxide Dismutase (SOD). We decided to estimate oxidative stress in the blood of patients suffering from vitiligo using SOD, MDA and GPX as markers for oxidative stress.

Materials and Method

This prospective cross sectional study was conducted in the Department of Dermatology, Venereology and Leprosy and Department of Biochemistry of our tertiary care hospital in Central India after approval from institutional ethics committee. A total of 40 patients were included with an informed consent. The inclusion criteria were all clinically diagnosed cases of Vitiligovulgaris, all patients >15years of age of either sex and untreated or treatment-naïve patients or those patients who have not received systemic treatment in the last 3 months for vitiligo or topical treatment in the last 2 weeks. The exclusion criteria were patients of segmental or localized vitiligo, patients on antioxidant medications and/or immune-suppressive during the last 3months for vitiligo, pregnant a lactating females and patients who have any concomitant systemic or dermatological disease.

Detailed history was taken and clinical examination was done in all subjects of the study group. Venous blood samples were collected for estimation of MDA, SOD and GPx.

Estimation of MDA: MDA was measured by spectrophotometry method. The method was based on the fact that lipid peroxide condenses with 1-methyl 2 phenyl indole (MPI) under acidic conditions resulting in the formation of red chromophore.

Estimation of superoxide dismutase (SOD): Superoxide dismutase (SOD) assay in whole blood samples was done according to 1983 Randox Kit by Williams J.A et al.[3] This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity is measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of the reduction of INT under the conditions of the assay.

Estimation of glutathione peroxidase (GPx): Assay of Glutathione Peroxidase (GPx) in whole blood samples was done by the Paglia and Valentine method[4] using Randox kit as per manufacturer's instructions. Glutathione peroxidase (GPx) enzyme catalyzes glutathione (GSH) oxidation by the enzyme cumenehydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH the oxidised Glutathione (GSSG) is immediately converted to the reduced form along with concomitant oxidation of NADPH to NADP+.

Blood levels of MDA, SOD, GPx and normal ranges as reported by the manufacturers are 0.8-1.3 nmol/ml, 164-240 u/ml and 4171-10881 u/l respectively. Elevated MDA indicated presence of oxidative stress while decreased levels of SOD and GPx indicated presence of oxidative stress. Data are expressed as mean ±SD. The Chi square test and p values are used for the interpretation of results in which *P* value of less than0.05 is considered as statistically significant.

Results

In our study, a total of 40 patients were enrolled. Out of these 40 patients a sample was hemolyzed in 4 patients. A data of 36 patients was analyzed. A total of 18 patients (50%) were females and 18 patients were males (50%).

Majority of vitiligo cases belonged to younger age groups. A total of 60% cases were in the age group of 16–25 years followed by 20% in 26–35years. The most common type of vitiligo seen clinically is Vitiligo vulgaris (75%) followed by 25% of acral vitiligo. Leuokotrichia was seen in 26% cases. A total of 50% cases were having active or unstable disease while 50% cases were having stable disease.

As assessed by MDA, 35/36 (97.22%) was found to have raised oxidant stress. As assessed by GPX, all i.e. 36/36 patients were (100%) were found to have raised oxidant stress. As assessed by SOD, 34/36 (94.44%) were found to have raised oxidant stress. Mean values of MDA in our patients were 4.3±2.75SD nmol/ml. Mean values of GPx in our patients were 2864±1008SD u/l. Mean values of SOD were 97.11±91.46SD u/ml. Mean values of MDA, GPX and SOD in matched controls were 0.91±0.21SD nmol/ml, 6058.60±1694SD u/l and 189.43±23.57SD u/ml respectively.

Discussion

In our study, we observed that the most common age group affected by active vitiligo is 16–25years. There was no male or female preponderance observed in our study. However the study was hospital based and involving lesser number of patients as compared to studies done earlier. Shajil et al[1] and Danesh Pazhooh et al[2] noted female preponderance in vitiligo. Vitiligo vulgaris was found to be the most common clinical type of vitiligo observed in our study.

Oxidative stress is induced by Reactive oxygen species (ROS) generation. Excess production of ROS and insufficient protection by anti-oxidants results in tissue damage including that of melanocytes. It is also proposed that they may modify melanocyte antigens to trigger autoimmune response.[5]

We observed significantly higher levels of MDA in our patients indicating oxidative stress in 92.22% of cases of vitiligo. Our study indicates that MDA levels are elevated in patients of leukoderma as compared to controls. MDA is an end product of lipid peroxidation. Results of our study are in concordance with previous studies on estimation of MDA levels in patients of vitiligo.[6-9] All of these studies are done in Indian population suffering from vitiligo. Results of our study and that of previous studies confirm MDA as a reliable marker for increased oxidative stress. With regard of MDA levels in subtypes of vitiligo, there was no statistically significant difference. Our study measured serum levels of MDA. Shin et al suggested use of erythrocyte MDA as more reliable and accurate marker for measurement of oxidative stress. They proposed that serum MDA levels are too low to be reliable in many cases and erythrocyte MDA reflect more accurately the oxidative stress in patients of vitiligo.[10]

Superoxide dismutase (SOD) is an important antioxidant enzyme which converts the pro-oxidant superoxide into H_2O_2. SOD, an antioxidant enzyme catalyzes the dismutation of superoxide anion ($O2^-$) into $O_2$and H_2O_2. In oxidative stress, to scavenge superoxide anions, SOD is increased and catalase is reduced. It is not clear whether SOD levels are elevated as a result of increased activity or decreased as a result of increased consumption in antagonizing oxidative stress. Results are therefore conflicting in various clinical studies that had been done in the past. Some studies done in the past have shown raised whole blood SOD levels. A case control study of 100 patients of vitiligo showed significantly higher levels in unstable or active vitiligo patients (90%) as compared to controls while in stable vitiligo a total of 92% had normal SOD levels.[11] This study showed that oxidative stress is likely to be involved in the aetiopathogenesis of vitiligo, as indicated by the high levels of serum superoxide dismutase activity. Sample size in this study

was comparable to that of our study. Some studies have shown raised erythrocyte SOD[12] or raised SOD in vitiligenous skin.[13]

Results as shown in our study showed decreased levels of SOD as compared to controls. This is similar to findings by Khan et al who reported that SOD levels are significantly lower in 30 patients of vitiligo as compared to controls.[6] Findings of low levels SOD in the blood was comparable to low levels of SOD in our study done on 40 patients of vitiligo. Koca R et al also found increased levels of MDA and low levels of SOD in 27 patients of vitiligo.[14] More studies are therefore needed to exactly know whether SOD are indeed increased due to enhanced activity or decreased due to enhanced consumption in neutralizing superoxide ions.

Glutathione peroxidase was found to be reduced in our study. Studies done in the past reveal inconsistent pattern in the level of GPx. Increased level of GPx is reported by Passi et al[15] whereas Agrawal et al reported decreased levels in vitiligo cases.[16] Hazneci E et al reported increased levels of erythrocyte GPx.[17] Zedan H et al reported low levels of Gpx in patients of vitiligo as compared to controls.[18] GPx is an antioxidant enzyme like SOD which prevents oxidative damage to the skin of vitiligo patients as a result of disturbed oxidant-antioxidant system. Trends of decreased SOD and GPx and increased MDA are similar to study done by Karsali N et al[19] who studied pre-treatment oxidative stress in patients of vitiligo. In this study after NB-UVB phototherapy, MDA was found to be reduced and SOD and GPX were found to be elevated indicating role of phototherapy in reducing oxidative stress.

Conclusion

In summary, blood estimation of MDA, SOD and GPX is relatively easy and simple to perform diagnostic tests to assess status of oxidative stress in patients of vitiligo. As compared to controls, increased MDA levels and reduced GPX and SOD levels were suggestive of oxidative stress in patients of vitiligo in our study. More studies of relatively larger sample size are required to further confirm these variations of MDA, SOD and GPx in the blood.

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