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ESTIMATION OF SERUM COPPER, SUPEROXIDE DISMUTASE AND REDUCED GLUTATHIONE LEVELS IN MELASMA: A CASE CONTROL STUDY

Dermatology

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ABSTRACT

BACKGROUND: Melasma is a difficult to manage hyperpigmentary disorder affecting facial skin.Studies have suggested the role of Ultraviolet Radiation induced reactive oxygen species leading to increased melanin pigment synthesis.Superoxide dismutase, a potent antioxidant enzyme and Reduced Glutathione, an antioxidant tripeptide act on oxidative pathway to render the reactive oxygen species harmless.Copper is thought to play a key role in melanogenic pathway as cofactor of tyrosinase enzyme.Oral as well as topical antioxidants are being used for the treatment of melasma with substantial evidence for their effectiveness.However the direct evidence is still lacking.

METHODS: A case-control study on 75 melasma cases and 75 healthy controls was conducted.Assessment of colour, pattern, type of melasma was done in each case.Superoxide Dismutase (SOD), Reduced Glutathione (GSH) and serum Copper levels were estimated in melasma patients and compared with healthy controls.

RESULTS: The subjects ranged from 18-52 years of age with female preponderance (Male: Female = 1:12).Mean duration of the disease was 2.12 years and mean MASI was 15.87±5.81.Mean levels of Serum copper(ug/dl), SOD(units/ml) and GSH(mg%) in cases were 127.79±16.14, 6.10±0.46 and 39.58±4.29 respectively compared to 126.32±14.61, 4.71±0.53 and 55.69±3.95 in controls.

CONCLUSION: Statistically, the difference in SOD and GSH levels between melasma cases and controls was found to be significant indicating definitive role of oxidative stress but serum copper levels was non-significant.

KEYWORDS

Oxidative stress, Melasma, Superoxide dismutase, Reduced glutathione, Melanogenesis, Hyperpigmentation, Tyrosinase

INTRODUCTION

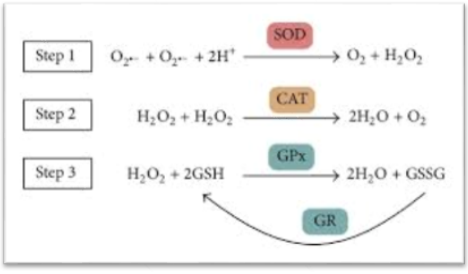
Melasma is an acquired disorder of hyperpigmentation with female preponderance, characterized by circumscribed macules and patches located chiefly over the sun exposed areas of face.[1]It is prevalent worldwide among different populations with a higher incidence in darker-complexioned individuals (skin types IV–VI) exposed to intense UltraViolet (UV) radiation.[2] Hypermelanosis is seen characterstically on the face, over centrofacial, malar and mandibular area involving additional sites such as extensor aspect of foreams, mid upper chest and neck rarely.[3,4]

Etiology is multifactorial with exacerbation seen after sun exposure. Other factors incriminated include genetic factors, pregnancy, oral contraceptives, estrogen progesterone therapies, thyroid dysfunction, certain cosmetics, topical agents, heavy metals and systemic medications.[5,6]

Metals known to induce or accelerate melanogenesis are copper, iron, bismuth, arsenic, silver and gold.[7]Melanogenesis is a complex process initiating with hydroxylation of tyrosine to dihydroxyphenyl alanine (DOPA), followed by dehydrogenation of DOPA to the corresponding quinine.Copper acts as a cofactor for tyrosinase and acts on first two major steps of melanin synthesis.[8]The activity of tyrosinase is thus thought to depend upon the amount of copper in blood.[9]

The level of copper is affected by diet and administration of hormones like estradiol, testosterone, pregnancy and chronic diseases.[10]In the past, various studies have been conducted to estimate serum copper levels in hypo as well as hyperpigmentary conditions with no conclusive evidence.

Few studies recently have come up with the role of Ultra-Violet (UV) radiation exposure leading to oxidative stress and increased melanin pigment synthesis.[11]Superoxide dismutase(SOD), Catalase and Glutathione peroxidase(GSH-Px) are intracellular enzymatic anti-oxidants and reduced glutathione is a potent antioxidant utilized in these reactions.Role of all these antioxidant enzymes is to scavenge the free radical i.e. superoxide ion (O_{2^-}) as follows.[12,13]



Melasma has attained special consideration due to cosmetic concerns to the patient and the therapeutic challenge it poses. Thorough history to rule out other causes and avoidance of triggers is advised.Sun protection and topical therapies such as hydroquinone, azelaic acid, retinoids, glycolic acid, kojic acid, ascorbic acid, topical steroids, cryotherapy, chemical peeling and laser treatment are used alone or in combination for treatment purpose.[14]

Among different modalities used based on varied etiopathogenesis, recently conducted studies show the effectiveness of anti-oxidants in melasma.[11,15] However, to best of our knowledge there are only a few studies evaluating the direct role of anti-oxidants in the etiopathogenesis of melasma.

Thus we aimed to evaluate risk factors in melasma evolution and to assess the levels of serum copper and oxidative stress in etiopathogenesis of melasma.

MATERIAL & METHODS

This case-control study was carried out in the department of Dermatology in association with department of Biochemistry of Guru Nanak Dev Hospital attached to Government Medical College, Amritsar, Punjab, India after obtaining the approval of the ethical committee of the institution. The study group consisted of 75 clinically diagnosed patients of melasma presenting to outpatient department of Dermatology from January 2014 to July 2015 after obtaining informed consent.

The exclusion criteria included patients having chronic liver–kidney disease, coronary heart disease, diabetes, deranged lipid profile, thyroid abnormalities, pregnant & lactating females. Patients who were cigarette/bidi smokers and those who had taken glutathione and antioxidants for the last three months were also excluded. Similarly age and gender matched 75 healthy volunteers were chosen as control group.

Detailed history regarding disease onset and progression as well as aggravating factors was taken as per the prescribed proforma. Clinical classification of melasma was done along with Melasma Area and Severity Index (MASI) score calculation.

Seven ml of venous blood was taken from healthy controls and melasma patients in dry disposable syringe under aseptic conditions by vein puncture in anticubital vein, in a dry sterile and acid washed vial for biochemical analysis.The collected blood was divided in 2 sets of vials to assess different biochemical parameters as follows:

1. For assessing Superoxide Dismutase activity and Serum Copper levels, the blood was allowed to stand for half an hour, after the clot formation the blood sample was centrifuged at 3000 rpm for 10 minutes and serum was taken for investigations.
2. For reduced glutathione (GSH), the whole blood sample was required, so the blood sample was put in heparinised vials.

-Serum Copper levels were estimated using standardized kits manufactured by Coral Clinical Systems.

Copper + Di-Br-PAESA → (acidic medium) Colored complex

Normal values (in serum)
(Males) : 80-140 µg/dl
(Females) : 80-155 µg/dl

Evaluation of biochemical parameters:

- SOD was estimated by the method of Marklund and Marklund, 1974 modified by Nandi and Chatterjee, 1988.[16]
- GSH levels were estimated by the method of Beutler et al,1963.[17]

Statistical analysis:

The results were expressed as mean ± standard error of mean.The counts were analyzed by chi-square test.The comparison was done by two-independent sample t-tests.*P* values < 0.05 were considered statistically significant.

RESULTS

Both patient and control group included 67 women (90%) and 8 men(10%) each.Majority of the patients i.e. 56(75%) were from reproductive age group i.e. 18-45years of age and 50(67%) were married.Majority of the melasma cases were housewives followed by students.

History of severe(4-6hours/day) and very-severe(>6 hours/day) sun exposure was present in 23(30.67%) and 9(12%) subjects from study cases and 13(17.33%)and 5(6.67%) controls respectively. Statistically this difference was significant(p value <0.05).

Forty-three melasma patients(57%) had history of cosmetics application in the form of over the counter fairness creams, topical steroids and unspecified moisturizing agents and 6 had history of oil application (mustard oil in 4, unspecified ayurvedic oil in 2).History of copper containing Intra-Uterine Device(IUD) insertion was present in 3(6.52%) melasma cases and 2(4.2%) controls out of the 46 married female melasma cases and 48 controls. Statistically this difference was non-significant. Family history of melasma in first degree relatives was found in 8(10.67%) melasma cases and 5(6.67%) controls which again was statistically non significant (p >0.05).

Skin types of the patients were diverse with majority i.e. 63(84%) had skin type IV followed by 7(9.33%) with skin type V and 5(6.67%) with skin type III. Duration of melasma ranged from 2 months to 8 years with mean duration being 2.12 years. Twelve patients(16%) had taken oral contraceptives followed by antihypertensives in 4(5.33%), anti-acne medication in 5(6.67%) and antidepressants in 2(2.67%). Nature, dose and duration of these medications was not specified. History of summer exacerbation was found in 16(21.33%) patients. History of melasma in previous pregnancies was present in 18 patients(40%) out of 36 married females.

Mixed color of pigmentation was seen in majority i.e. 26(34.7%) followed by light-brown in 23(30.7%), dark-brown in 15(20%) and bluish in 11(14.6%).Pigmentation was non uniform in 52(69%) and uniform in 23(31%).Centrofacial pattern of melasma was seen in 53(71%) (Figure 1) followed by malar in 18(24%) (Figure 2a and b) and mandibular in 4(5%) (Figure 3) while extrafacial pigmentation was seen in only 3(4%) patients, involving V area of neck and dorsal aspect of forearms.Mean MASI score was 15.87 ± 5.81with 31 patients(41.33%) having MASI ranging between 11-20.9 followed by 27(36%) with MASI >21 while 17(22.67%) had MASI ranging between 0-10.9.

Moderate and severe grade iron-deficiency anaemia was seen in 9(12%) and 5(6.67%) study cases respectively compared to 4(5.33%) and 1(1.33%) in controls.This difference was statistically significant (p value <0.05).Regarding serum copper levels, mean±SD of the copper levels in male patients with melasma was 121.25±5.70 compared to 121.85±9.91 in male controls whereas in female melasma cases, it was 132.96±20.88 compared to 130.78±19.31 in female controls.Statistically this difference was non-significant (p value >0.05) (Table 1).

On comparison of SOD levels, male patients with melasma had the mean±SD of SOD (in U/ml) of 5.96±0.40 while in male controls it was 4.87±0.51.In female melasma cases, it was 6.23±0.52 against 4.55±0.55 in female controls.This difference was highly significant (p value <0.05) (Table 2).

GSH levels in male patients with melasma had mean±SD (in mg%) of 40.35±3.98 compared to 55.73±3.41 in male controls.In female melasma cases, the levels were 38.83±4.60 compared to 55.66±4.48 in controls.This difference was highly significant (p value <0.05) (Table 3).

DISCUSSION

Melasma is a globally widespread disorder of hyperpigmentation with widely debatable etiology. Multitude of cosmetic procedures are available boasting to treat it. But still there is dire need to understand the etiopathogenesis of melasma with risk factor evaluation so as to find the best therapeutic and preventive option to improve cosmetic appeal and psychosocial life of the patients. In our study we tried to assess the risk factors associated and the role of oxidant- antioxidant defense of the body in the etiology of melasma.

Higher incidence of melasma (75%) in the reproductive age group with median age of 32 years with female preponderance (male to female ratio of 1:12) was seen. Forty percent of the cases involved were housewives who did not had much photo exposure but still constituted majority of our patients and higher incidence was seen among married females.[18,19,20,21,22,23]All these findings support the role of hormonal factors in etiopathogenesis of melasma. Positive family history of melasma was seen in 11% of the cases under our study as seen in previous studies.[19,20]History of the onset or aggravation of melasma during previous pregnancy in 18(40%) out of 46 married female cases and OCPs intake history in upto 16% cases further supports hormonal influences probably through estrogen receptors present on melanocytes which become hyperactive in presence of excess estrogen leading to increased melanin production.[24]More engagement in outdoor activities as well as seeking treatment early due to greater cosmetic concern in student group was seen. Increased incidence in beauticians suggest the possibility of self medication in the form of unspecified beauty products which can act as a triggering or aggravating factor.

Significant sun exposure history indicates enhanced melanin synthesis and melanocyte proliferation as well as cell migration on UV exposure. Melasma skin have been found to show the features of prominent solar damage and solar elastosis on immunohistochemical studies affecting the development of hyperpigmentation.[22,23]Seasonal variation with summer exacerbation in 21% melasma patients can be attributed to prolonged day duration as well as higher intensity of UVR in summers. Perfume agents and unspecified creams used in upto half of melasma patients indicate the role of triggering/ contact sensitizing agents in melasma.[19,20]Topical application of mustard oil, known to contain allyl isothio-cyanate, a contact sensitizer, was present in only 5% of the melasma patients.Previous studies have shown such association.[23]

Theoretically slow release of copper from Cu containing IUD is thought to exacerbate melasma via increasing the activity of tyrosinase enzyme which uses copper as a co-factor but like previous studies no direct effect of serum copper in melasma etiopthogenesis was found.

Non-uniform pattern of pigmentation seen in majority indicates the influence of vast array of risk factors determining pigment localization. Centrofacial pattern was most common pattern supporting previous observations and indicate persistent nature of the condition and progression leading to involvement of more area over face.Also hormonal factors are known to cause pigmentation more over the central areas of the face. Extrafacial pigmentation was typically located over photoexposed areas.

Though majority of Indian female population is iron deficient, significant variation in the incidence of moderate and severe anemia in melasma cases and controls proposes a definitive role of Iron deficiency anemia. However serum copper levels did not reveal any association as observed in previous studies. It suggests mutifactorial basis of pigmentation rather than sole dependence on melanin production.[13]

Oxidative mechanisms influencing melanin pathway are chiefly determined by activity of antioxidant enzymes i.e. SOD, Catalase, GSH Peroxidase and GSH Reductase. Glutathione in its reduced form acts as an important substrate which is utilized in neutralization reactions by these enzymes. SOD is the primary enzyme clearing free oxygen radicals by converting superoxide anions to hydrogen peroxide which is then further detoxified.[25]Higher SOD activity in melasma cases reveal higher level of oxidative stress, to combat which antioxidant defense mechanisms become hyperactive.Significantly lower levels of GSH in the melasma cases thus indicates its increased utilization in detoxification reactions.[23]

However levels of SOD and GSH compared in relation to disease duration, color, clinical type of melasma and MASI score were insignificant attributed to multifactorial etiopathogenesis.

We observed that sun exposure, history of melasma in previously in pregnancies, topical and cosmetic agents, hormonal dysfunction, OCPs intake and iron deficiency anemia were risk factors associated with higher incidence of melasma.Markedly raised SOD activity and significantly decreased GSH levels in melasma patients indicate higher oxidative stress in melasma however serum copper levels were not found to have any impact on melasma pathogenesis.

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Ethical approval: None required

Table 1. Comparsion of Serum Copper levels (µg/dl) in the study cases and controls

Sex	Study cases		Controls		'p' value
	No of Subjects	Mean ±SD	No of Subjects	Mean ±SD	
Males	8	121.25+5.70	8	121.85 + 9.91	0.882 (NS)
Females	67	132.96 + 20.88	67	130.78 + 19.31	0.36 (NS)

a sterisk(*) indicates the significance of 'p' value. (*significant, **Highly significant)

Table 2. Comparison of Superoxide dismutase levels (SOD in Units/ ml) in the study cases and controls.

Sex	Study cases		Controls		'p' value
	Number of Subjects	Mean ±SD U/ml	Number of Subjects	Mean ±SD U/ml	
Males	8	5.96+0.40	8	4.87 + 0.51	0.03*
Females	67	6.23 + 0.52	67	4.55 + 0.55	0.000**

a. asterisk(*) indicates the significance of 'p' value. (*significant, **Highly significant)

Table 3. Comparison of Serum Reduced Glutathione (GSH in mg%) in the study cases and controls.

Sex	Study cases		Controls		'p' value
	Number of Subjects	Mean ±SD	Number of Subjects	Mean ±SD	
Males	8	40.35+3.98	8	55.73 + 3.41	0.001**
Females	67	38.83 + 4.60	67	55.66 + 4.48	0.001**

a. asterisk(*) indicates the significance of 'p' value. (*significant, **Highly significant)

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