ORIGINAL ARTICLE

Assessment of Circulating Biochemical Markers and Antioxidative Status in patients suffering from Melasma

NAVEED SHUJA[1], UMAIR MANSOOR BAJWA[2], SABA IQBAL[4], *FAUZIA HAMEED[2], ABDUL MANAN[2], ATIYA FATIMA[2], AMNA MAHMOOD[2], SAIMA ZAHEER[2], ARIF MALIK[2], KAMRAN AZIZ[1], NAUREEN RUBY[3]*

ABSTRACT

Human phenotype including skin colour patterns is associated with genetic, evolutional as well as cultural aspects which rely on production of melanin. Melasma is crucial disorder related to the skin that affects the population. The aim of this study was to evaluate the various contributing circulating biochemical markers that could be responsible for melasma. Fifty (50) patients suffering from melasma and twenty (20) clinically healthy individuals were selected for this study. Lipid profile as well as oxidative and anti-oxidative profiles was estimated in patients and healthy individuals. HDL was decreased in patients (1.39±0.10*) as compared to control (1.73±0.17) group. On contrary, TCh (5.48±0.92*), Tg (2.59±0.53*), and LDL (2.48±0.23*) were raised in patients. Alterations in lipid profile were statistically significant (P<0.05). Malondialdehyde (MDA) was elevated (5.44±1.14*) in patients while anti-oxidative parameters were decreased in patients and all were statistically significant (P<0.05). It can be concluded that not only the melanin but also other circulating biomarkers like cholesterol, Tg, HDL and LDL may be the contributing factors for the progression of melasma. Moreover, oxidative stress markers may also be used in disease prognosis and diagnosis.

Keywords: melasma, melanin, anti-oxidative, malondialdehyde, cholesterol, HDL, LDL, Tg

INTRODUCTION

Melasma is an important disease frequent in general population that greatly affects the human phenotype. The skin color patterns are related to the genetic, evolutional and cultural aspects which depend upon the production of melanin, the number and distribution of the cytoplasmic particles called melanosomes, the storage structure of melanin (Costin and Hearing, 2007). Higher the concentration of melanin, the darker is the skin (Jones et al., 2002). Other exogenous and endogenous pigments like carotenoids, red oxygenated hemoglobin and blue reduced hemoglobin in the capillary beds of dermis also play a vital role in the development of skin color (Miot et al., 2007 and Bolognia et al., 2003).

The density of melanocytes is relatively constant in different races, about 2000 per square millimeter in the skin of head and forelimbs and about 1000 in other body parts. The distribution of melanocytes in epidermis is regulated by keratinocytes (Sulem et al., 2007). The phenotypical difference between different races does not lie upon the number of melanocytes neither on the production rate of melanin but predominantly the quality of melanosomes. Mature and larger melanosomes present in the form of units appear in black people while clustered immature melanosomes are apparent in white people (Jablonski and Chaplin, 2007).

Synthesis of melanin depends upon many genes.Malfunctioning or mutation in these genes causes the formation of dark reddish brown macules with discontinuous contour on the skin areas more exposed to the light more frequently on the cheeks, eyelids, chin, forehead and forelimbs (Sulaimon and Kitchwell, 2003). There are many pathophysiological factors which are responsible for hypermelanosis which trigger multiple signaling pathways in both melanocytes and keratinocytes. Ultraviolet radiation triggers the activation of p53 gene through DNA damage in the nucleus of keratinocytes (Lin and Fisher, 2007).This increases the modulation of gene encoding the propiomelanocortin (POMC) (Abdel et al., 2001).

Posttranscriptional modification of POMC stimulates the production of melanocyte stimulating hormone (MSH) and β-endorphin (Biossy, 1988 and Rouzand et al., 2005).MSH is transported to the melanocytes where it binds to the melanocortin 1 receptor (MCR1) which activates cAMP response element binding protein (CREB) through downstream stimulation of cAMP (Storm and Elder, 2006 and Murisier and Beermann, 2006). The melanocyte master regulator MITF is activated further channelizing the transcription and modification of melanogenic enzymes with tyrosinase, thereby

[1]*Biochemistry Department, Lahore Medical & Dental College (LMDC), Lahore, Pakistan.*
[2]*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan.*
[3]*Govt. Ayesha Post Graduate College, Lahore, Pakistan.*
4Deptt. Of Physiology, University College of Medicine & Dentistry, University of Lahore
Correspondence to Dr. Arif Malik, Associate Professor Email: arifuaf@yahoo.com Cell: 0321-8448196

inducing the production of melanin pigment which is actively transported from the melanocytes in the dermis to the keratinocytes located in the epidermis where the pigment vesicles accumulate over the photo exposed surface of the nucleus subsequently causing tanning as a consequence (Thody and Graham, 1998).

MATERIALS AND METHODS

Total seventy subjects were selected for the study including twenty age and sex matched clinically healthy individuals and fifty patients suffering from melasma. Details of clinical complications as known HCV or HBV, alcohol intake, diabetes mellitus or any other were collected from all subjects. Glutathione, superoxide dismutase, catalase, MDA, vitamin C and E were measured by the method of Moron *et al*., 1979, Kakkar, 1972, Aebi, 1974, Ohkawa, 1979, Mohammad, 1991 and Kayden *et al*., 1973 respectively. Results were analyzed statistically by independent t-test at $p<0.05$.

RESULTS

The previous literature deliberately illustrates that melasma presents a manifestation of metabolic syndrome and multiple biochemical markers are affected in this malfunction. The present study shows the changed BMI status, lipid profile, hematological, stress biomarkers and fasting blood glucose profiles. Raised BMI has been observed in melasma group having a mean value of 36.76kg/m^2which is statistically significant increase as compared to control group with BMI 21.55 kg/m^2. The hematological profile of melasma patients also showed distinctive results when compared with normal control group. Hemoglobin of 14.74mg/dl was decreased to 12.8mg/dl in pathogenic condition. Similarly, RBCs count was also decreased from 6.07x10^6 mm3to 3.45x$10^6$$mm^{3.}$ The lipid profile including total cholesterol (TCh), triglycerides (TG), low density lipoproteins (LDL) and high density lipoprotein (HDL) of melasma verses control group differ significantly at P value of 0.05. A declining trend was observed for serum HDL levels from 1.74 mmol/l to 1.39 mmol/l in control and melasma group respectively however an increasing trend was noticed in case of TG, LDL and TCh. In melasma group TG level of 2.59 mmol/l is not significantly higher than control group (1.2mmol/l) but also much elevated as compared to normal range (0.2-1.3 mmol/l). The biochemical stress markers profile also exhibited significant increase in MDA and NO from 1.36nmol/ml and 11.28 µmol/l respectively in control group to 5.44nmol/ml and 15.5µmol/l for MDA and NO respectively in melasma group.Reverse is true for both endogenous and exogenous antioxidants. All antioxidants decrease in melasma group as compared to control group. Fasting blood glucose level is also shown to be significantly increased from normal 100mg/dl to 115.9mg/dl in melasma suggesting its significant role in melasma progression.

Table 1: Alterations in various circulating biochemical markers in melasma

Parameters	Control	Melasma	P value
BMI	21.55±1.57	36.76±7.71	0.000
Hb	14.75±1.08	12.81±1.09	0.000
RBC	6.07±0.45	3.45±0.56	0.000
TCh	4.44±0.37	5.48±0.92	0.000
TG	1.24±0.15	2.59±0.53	0.000
LDL	2.31±0.15	2.48±0.23	0.002
HDL	1.73±0.17	1.39±0.10	0.000
MDA	1.36±0.38	5.44±1.14	0.000
SOD	0.73±0.25	0.24±0.11	0.007
GSH	9.77±1.17	4.91±1.11	0.000
Catalase	4.27±0.73	1.43±0.35	0.000
NO	11.28±1.35	15.50±1.64	0.000
Vitamin E	0.29±0.067	0.22±0.073	0.000
Vitamin C	0.57±0.08	0.33±0.07	0.000

BMI=body mass index; Hb=hemoglobin; RBC=red blood corpuscles; TCh=total cholesterol; TG=triglyceride; LDL=low density lipoproteins; HDL=high density lipoproteins; MDA=malondialdehyde; SOD=superoxide dismutase; GSH=reduced glutathione; NO=nitric oxide

DISCUSSION

Various internal and external factors influence melanogenesis (Wagner et al., 2002). The most abundant thiol in the body known to be is glutathione that has a great skin lightening property by preventing the formation of melanin from tyrosine by tyrosinase (Hearing, 2005). Thiols with copper sulfate inactivate the active site of tyrosinase by chelating copper ions irreversibly (Boissy, 2003). However, pK value of glutathione in serum found to be 6x10^4 m has no effect on tyrosinase activity. The quenching of free radicals and peroxidesby GSH contributes to its anti melanogenic activity (Szabo et al., 1988).

Cysteine and glutathione causes lighter skin pigmentation through pheo-melanogenesis by reacting with dopaquinone. However, when thiols level drop down, dopaquinone is converted to indole derivatives resulting in eumelanin formation (Rouzaud and Hearing, 2005). Glutathione conjugates with dopaquinone through glutathione-S-transferase to form glutathionyl-dopa which upon enzymatic hydrolysis through glutamyl-transpepetidase is converted to cyteinyldopa ultimately entering the pheao-melanogenic pathway (Slominski et al., 2004). Highly toxic chemicals like quinones are melanotoxic which destroy the

melanocytes resulting in lighter skin pigmentation (Klaus and Snell, 1967). Glutathione due to its antioxidative property prevent the melanocytes from such oxidation reactions (Prusis et al., 1997).

A case study in mice indicated the both BSO and cystamine aggrevated the depigmenting effect of hydroquinone on black and yellow hairless mice by depleting GSH in their cells (Abdel et al., 2000). N-acetyl-4-S-CAP is a competitive inhibitor of tyrosinase which creates cytotoxicity of orthoquinone intermediates. The most potent inhibitor of glutamyl-S-transferase, all-trans-retinoic acid (ATRA) depletes the intercellular GSH synergistically by increasing the epidermal turnover resulting in enhanced depigmenting activity of hydroquinone and hydroxyanisole (Voisey et al., 2003 and Prusis et al., 1995). It is also reported that some antimelanogenic chemicals like 4-Tertiary butyl catechol (TBC) increase the levels of glutathione reductase and gamma glutamyl-transpepetidase promoting pheo-melanogenesis (Valverde et al., 1995).

Immunohistochemical findings suggests that α-Melanocyte stimulating hormone (MSH) stimulate the immune system in affected skin area by stimulating melanocortin 1 receptor (MCR1) responsible for the genesis of melasma which intimate its potent role in hyperpigmentation called melasma (Rees, 2000). Some other factors like β- estradiol increases the expression of estrogen receptors in skin by over expressing α-MSH and MC1-R in melanocytes promoting physiopathogenesis of melasma (Tan et al., 1999).These experimental evidences support for the future research to investigate the possible the treatments of melasma by using the above discussed enzymes like GSH, BSO and others in order to conduct randomized, double blind clinical trials in humans for the effective treatment of melasma (Rouzaud et al., 2006, Schaffer and Bolognia, 2001).

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