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*Full Length Research Paper*

# Serum activities of anti-oxidant enzymes and possible involvement of genetic factor in androgenetic alopecia in male Nigerian subjects

**Iyanda A.A.**

Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo.
E –mail: lapeiyanda@yahoo.com

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**Various environmental factors which prone an individual to oxidative stress have been linked with androgenetic alopecia (AGA) but the result outcome of various studies emanating from different regions has not consistently confirmed such association, an indication that there may be race involvement. The aim of this study therefore is to identify using antioxidant enzymes as indices of study if AGA is oxidative stress-induced in Nigerian subjects exposed to cigarette smoke and alcohol. Androgenetic alopecia subjects exposed to cigarette smoke, alcohol consuming or non-smoking/non-alcohol consuming were used for the study with each group comprising 30 subjects while 40 subjects served as the control. Serum activities of catalase, glutathione peroxidase and superoxide dismutase were estimated, data were also obtained on age, durations of alopecia, smoking and alcohol consumption as well as family history of AGA (maternal and paternal). Results revealed that serum activities of both catalase and superoxide dismutase were significantly different ($p<0.05$) in the smoking and alcohol consuming group compared with control while that of glutathione peroxidase was significantly different in smokers ($p<0.05$) but not in alcohol consuming group ($p>0.05$). An analysis of family history showed that greater than 75% of the subjects had family history of AGA. Moreover, correlation study identified a relationship between smoking and duration of alopecia and two of the antioxidant indices, although alcohol consumption was positively correlated with superoxide dismutase but there was no correlation between it and alopecia. Our findings suggest that both smoking and family history of alopecia but not alcohol consumption seem to play a role in the pathogenesis of alopecia and that smoking-induced AGA may be oxidative stress induced.**

**Keywords:** Serum activities, anti-oxidant enzymes, genetic factor, androgenetic alopecia, male Nigerian.

## INTRODUCTION

Androgenetic alopecia (AGA) is probably the most common cause of male pattern baldness (MPB) in all races (Pathomvanich et al., 2002) as well as most common hair loss disorder found in male and female subjects. It is known to be more common in males than females (Blume-Peytavi et al., 2011; Norwood, 1975) AGA has been identified to be androgen dependent and closely linked to inheritance mode of gene polymorphisms (Hoffmann, 2002). Specifically Ellis et al. (2002) have identified that the gene which encodes the androgen receptor plays a role in the pathogenesis of AGA. Results of the study of Ellis et al. (2002) further

revealed that not only androgens but a genetic predisposition, seem to be prerequisites for the manifestation of AGA.

Multifactorial causes of AGA was confirmed through observations from clinical practice which revealed that by simply blocking androgens, the process of AGA was not abolished since conversion of miniaturized follicles to terminal ones in advanced alopecia did not occur (Trüeb, 2002). This points to a probable involvement of other factors presumably environmental ones. Association between smoking and AGA has been investigated in some past studies (Mosley and Gibbs, 1996; Matilainen et al., 2003; Severi et al., 2003), with inconclusive result. A study carried out among the Asians has identified a possible association between smoking and androgenetic alopecia while another carried out in Australian subjects showed a lack of correlation between both factors (Severi et al., 2003) an indication that a possible role of cigarette smoke on the process of hair loss may have a race bias. This may not be surprising because race is a major factor accounting for the differences in prevalence of androgenetic alopecia world-wide.

Hoffmann (2002) has reported of higher prevalence of AGA in men of white race/ethnicity than the Asian, Native American, and African American men. Another study has also confirmed this; Pathomvanich et al. (2002) have indicated that the prevalence of male pattern alopecia in Asian subjects is lower than in Caucasian ones, being only about one-fourth to one-third on average compared to Caucasians, although a gradual increase in prevalence even among the Asian subjects is being experienced in recent past which these workers have attributed to changes in socioeconomic environment as well as westernized diet. Apart from a possible impact of cigarette smoke on the pathogenesis of androgenetic alopecia, the study of Severi et al. (2003) have indicated that alcohol consumption may be associated with AGA among the Australian subjects.

The aim of this study therefore is to investigate using a cross-sectional set up if smoking and alcohol consumption play a role in the pathogenesis of androgenetic alopecia in subjects of African descent. Moreover, since both smoking and alcohol consumption seem to increase the oxidative stress of an organism, through this study we hope to identify using antioxidant indices like catalase, glutathione peroxidase and superoxide dismutase if smoking related androgenetic alopecia exists and is oxidative stress mediated.

## SUBJECTS AND METHODS

### Subjects

A cross-sectional study designed to determine hereditary and oxidative stress as bases of AGA in different categories of subjects. The categories are smokers, alcohol consuming, and non-smoking/non-alcohol consuming androgenetic alopecia subjects, each category consists of 30 subjects and 40 subjects served as the control. These subjects were randomly selected within Ibadan metropolis. Diagnosis of AGA was based on the classification by Norwood; all AGA subjects recruited for the study had AGA of type III or above. In addition, all subjects were twenty years and above. None of the subjects were on any medication capable of inducing alopecia (e.g. anticonvulsants, antidepressants, angiotensin-converting enzyme inhibitors, and the anticoagulants heparin and warfarin), or had had history of any of the diseases (e.g. thyroid diseases, hepatic failure, chronic renal failure, psoriasis, inflammatory bowel disease, allergic contact dermatitis and seborrheic dermatitis) or conditions (e.g. starvation, malnutrition, malabsorption) capable of inducing alopecia. Subjects with any other type of alopecia were excluded from this study. Moreover, men with family history of any other type of alopecia were also excluded.

Subjects provided information on history of AGA on both maternal and paternal side, age at onset of AGA, number of cigarette sticks smoked per day, quantity of alcohol consumed, and other possible environmental risk factors. Types of hair grooming procedure and cosmetic materials were obtained e.g. type of cosmetic soap, type of hair cream, numbers of hair wash or grooming per week. All study procedures conformed to the principle outlined in the Declaration of Helsinki of 1975 (revised in 2000).

### Biochemical determinations

Ten millimeters of blood was collected from each subject between the hours of 9.00 h and 12.00 h, these blood samples were centrifuged for 10 minutes at 3000 r.p.m. to obtain serum which was stored at -20°C until they were required for analyses. The activities of serum catalase, glutathione peroxidase and superoxide dismutase were determined using the methods of Sinha (1972), Rotruck et al. (1973) and Kakkar et al. (1984). Hitachi 902 Automated machine (Roche Diagnostic, Germany) was used for these estimations.

### Statistical analyses

Statistical analyses were performed with the SPSS/PC software (Version 15.0 for windows SPSS Inc, Chicago, IL, USA). Student's t-test was used to test the level of significance between each of the test groups and control group, and the analysis of variance (ANOVA) to determine intergroup comparison, Pearson's coefficient was employed to establish correlations among variables, $p \leq 0.05$ was considered significant in all analyses.

**Table 1.** Age, duration of alopecia and activities of serum catalase, glutathione peroxidase and superoxide dismutase in two categories of alopecia subjects and controls

| | GROUP A Mean±SEM | GROUP C Mean±SEM | GROUP D Mean±SEM | F values | P values |
|---|---|---|---|---|---|
| Catalase (µmol $H_2O_2$ consumed/(min·mg protein)) | 2.11±0.09* | 2.40±0.07 | 2.46±0.07 | 4.636 | 0.012‡ |
| Glutathione peroxidase (µmol GSH consumed/(min·mg protein) | 8.87±0.15* | 9.44±1.00 | 9.51±0.15 | 6.662 | 0.002‡ |
| Superoxide dismutase (U/mg protein) | 11.53±0.24* | 12.40±0.16 | 12.52±0.16 | 7.823 | 0.001‡ |
| Age (years) | 43.03±1.88 | 44.47±1.91 | 42.30±2.06 | 0.319 | 0.728 |
| Duration of alopecia (years) | 12.50±0.85 | 8.80±0.60 | - | 12.560 | 0.001 |

Mean±SEM- mean±standard error of mean. *Significant at p≤0.05, using Student t test. ‡ Significant at p≤0.05, using ANOVA.

**Table 2.** Age, duration of alopecia and activities of serum catalase, glutathione peroxidase and superoxide dismutase in two categories of alopecia subjects and controls

| | GROUP B Mean±SEM | GROUP C Mean±SEM | GROUP D Mean±SEM | F values | P values |
|---|---|---|---|---|---|
| Catalase (µmol $H_2O_2$ consumed/(min·mg protein)) | 2.02±0.09* | 2.40±0.07 | 2.46±0.07 | 0.022 | 0.043‡ |
| Glutathione peroxidase (µmol GSH consumed/(min·mg protein) | 9.37±0.13 | 9.44±1.00 | 9.51±0.15 | 0.283 | 0.754 |
| Superoxide dismutase (U/mg protein) | 10.24±0.34* | 12.40±0.16 | 12.52±0.16 | 29.269 | 0.001‡ |
| Age (years) | 44.93±1.59 | 44.47±1.91 | 42.30±2.06 | 0.569 | 0.568 |
| Duration of alopecia (years) | 11.60±0.77 | 8.80±0.60 | - | 8.218 | 0.006 |

Mean±SEM- mean±standard error of mean. *Significant at p≤0.05, using Student t test. ‡ Significant at p≤0.05, using ANOVA.

**Table 3.** Correlation between age and duration of alopecia and activities of serum catalase, glutathione peroxidase and superoxide dismutase in two categories of alopecia subjects and controls

| | Duration of alopecia | Duration of smoking | Duration of alcohol consumption |
|---|---|---|---|
| Catalase | | | |
| GROUP A | r=-0.101;p=594 | r=-0.457;p=0.045* | |
| GROUP B | r=0.084;p=0.660 | | r=-0.120;p=0.528 |
| GROUP C | r=-0.327;p=0.077 | | |
| Glutathione peroxidase | | | |
| GROUP A | r=-0.112;p=0.554 | r=-0.269;p=0.160 | |
| GROUP B | r=0.055;p=0.772 | | r=0.074;p=0.669 |
| GROUP C | r=0.340;p=0.066 | | |
| Superoxide dismutase | | - | |
| GROUP A | r=-0.-0.079;t=0.678 | r=0.842;p=0.038* | |
| GROUP B | r=-0.108;t=0.569 | | r=0.369;p=0.045* |
| GROUP C | r=-0.144;t=0.548 | | |
| Duration of alopecia | | | |
| GROUP A | - | r=0.784;p=0.001* | r=0.196;p=0.298 |
| GROUP B | | | |
| GROUP C | | | |

## RESULTS

Results in Table 1 reveal that smoking caused significant decline in the activities of the antioxidant enzymes. Catalase, glutathione peroxidase and superoxide dismutase are significantly lower (p<0.05) in smokers compared with control while the non-smoking/non-alcohol consuming alopecia subjects have not shown any significant difference in the activities of these enzymes. In Table 2; serum activities of catalase and superoxide dismutase (p<0.05) but not glutathione peroxidase are significantly different in alcohol consuming group

compared with control.

Moreover, over seventy-five percent of all alopecia subjects confirmed that either maternal, paternal or both had family history of alopecia compared to approximately twenty-five percent of the control subjects. The average sticks of cigarette per person per day over the period of 10 weeks prior to the commencement of the study were 13. Less than 20% confirmed daily grooming of the hair using any type of hair grooming procedure involving either any of the cosmetic materials such as soap or hair cream. Although there is no significant difference in the age of subjects in the three alopecia groups compared with control, duration of alopecia is significantly different in smoking and alcohol consuming group compared with non-smoking/non-alcohol consuming group.

## DISCUSION

The result of our study in which over 75% of the subjects in the androgenetic alopecia (AGA) groups identified that either their father, maternal grandfather or both have or had manifested alopecia compared to approximately 25% in control group, is a finding which probably points to the involvement of hereditary in the pathogenesis of AGA. A number of workers have shown that a genetic predisposition is a common characteristic feature of AGA. In doing this, Braun-Falco and Bergner (1989) have revealed that the pattern of inheritance of AGA is consistent with an autosomal dominant transmission; while Bergfeld (1995) as well as Ellis et al. (1998) though confirmed genetic connection, but they have also indicated that the relatively strong concordance of the degree of baldness in fathers and sons though genetically based is not consistent with a simple Mendelian trait but rather a polygenic basis may be involved. Results of the study of Chumlea et al. (2004) also suggest that there is a possibility of male pattern hair loss being dependent on family history and age. Specifically, they identified that hair loss in a man's father also appears to play an important role in increasing a man's risk of hair loss, either in conjunction with a history of hair loss in the mother or hair loss in the maternal grandfather.

To further identify genetic involvement, is the observation made through some studies which shows that although the predisposing genes responsible for AGA are still not fully elucidated and the genes for type 1 and type 2 5alpha-reductase (5alpha-R)- enzymes responsible for the conversion of testosterone to dihydrotestosterone (DHT)- are not associated with the inheritance of AGA (Ellis et al., 1998; Sreekumar et al., 1999) the discovery that polycystic ovaries (PCO) in females and early onset AGA in brothers of those women are associated with one allele of the steroid metabolism gene CYP17, an enzyme which plays a role in the generation of androgen or its action (Carey et al., 1993; Ferriman and Purdie, 1979) may further lay credence to genetic involvement.

A link between a susceptibility gene for PCO and a polymorphism of the insulin gene has been described; AGA has also been associated with abnormal insulin action (Waterworth, et al., 1997). Sawaya and Shalita (1998), in shedding more light on this, have revealed that in man, mutations in the androgen receptor (AR) gene have clinical implications and shorter so called CAG-repeat lengths within the AR gene may be associated with the development of not only AGA but also androgen-mediated skin disorders in both men and women, but since AR gene is located on the X chromosome, this does not explain father-to-son inheritance which Ellis et al. (1998) have observed.

Although our study has a cross-sectional set up, the results of this study which show a positive correlation between smoking and AGA is in agreement with those of Su and Chen (2007) who also observed a positive association between smoking and AGA, although theirs was a population-based study. Moreover, results of our study were consistent with findings of Mosley and Gibbs (1996) who also using a cross-sectional survey in a general surgical outpatient clinic in the United Kingdom observed an association between smoking and AGA. Findings from two other studies (Matilainen et al., 2003; Severi et al., 2003) though have not identified smoking as a significant risk factor in AGA, Severi et al. (2003) have identified that a lower risk of AGA may exist among current smokers (odd ratio, 0.86; 95% confidence interval, 0.54-1.38) and ex-smokers (odd ratio, 0.91; 95% confidence interval, 0.65-1.29), although their results were not statistically significant. This may be because unlike our study different AGA classification categories instead of the Norwood classification were used.

Su and Chen (2007) and Trüeb (2003) have postulated that the mechanisms by which smoking induce hair loss may be multifactorial. Some of those mechanisms may include the following; that cigarette smoking may be harmful to the microvasculature of the dermal hair papilla and the different genotoxicants derived from cigarette smoke may also be dangerous to DNA of the hair follicle. In addition, imbalance in the follicular protease or antiprotease system as a result of smoking can also not be ruled out. Smoking-induced oxidative stress on the other hand may result in the production of proinflammatory cytokines that, in turn, results in follicular microinflammation and fibrosis.

This may be because the follicular miniaturisation that accompanies these hair cycle changes affects the papilla, the matrix, and ultimately the hair shaft. The dermal papilla is fundamental to the maintenance of hair growth (Oliver and Jahoda, 1989) and is probably the target for androgen mediated changes in the hair cycle and miniaturisation of the follicle (Obana and Uno, 1996). With reduced follicle size, the hairs they produce become finer (which have been estimated to be of mean diameter

of between 0.08 mm and <0.06 mm), and pigment production decreases (Rushton et al., 1991). It is important to note that the possible miniaturization which can occur as a result of smoking occurs either in early anagen or possibly catagen or telogen hairs, producing a stepwise reduction in size of the follicle with each successive cycle.

Finally, Trüeb (2003) has identified that cigarette smoking may yield a relative hypoestrogenic state by provoking an elevated hydroxylation of estradiol and inhibition of aromatase. The cytochrome P450 aromatase enzyme is required for bioconversion of androgens to estrogens. Aromatase has been detected in the external root sheath of anagen hair follicle (Sawaya and Penneys, 1992). Its presence in the external root sheath of anagen hair follicle is suggestive of the fact that aromatase probably has a function in the intrafollicular androgen metabolism by converting potent androgens to less potent estrogens in order to avoid potentially harmful androgen-mediated effects on androgen-dependent hair follicle. The fact that female subjects taking aromatase inhibitors for the treatment of breast cancer usually present with AGA-like hair loss (Ayoub et al., 1997) and that in both men and women aromatase activity has been shown to be diminished in hair follicle affected by AGA (Sawaya, 1991) is an evidence of the role of this enzyme in AGA.

Moreover since smoking has been describes as a factor capable of inducing significant oxidative stress, its impact on antioxidant is always depletory in nature, such that it causes drastic reduction in the levels of these antioxidants, many of which play important role in the physiology of the hair. For example, zinc as well as selenium helps in the proper utilization of proteins and hormones in hair formation, on the other hand, vitamins A, C, E and vitamin B6 act in conjunction with these two minerals to promote hair regrowth.

While genetic factors seem to play the principal role in the development and progression of androgenic alopecia, lifestyle (e.g. smoking and alcohol consumption) also likely plays a minor role as demonstrated by the vast increase in male and female pattern baldness in Japan subsequent to the second World War which has been linked to changes in lifestyle, especially as the country moved to a higher-calorie, higher-fat diet and a more sedentary lifestyle. These are conditions capable of inducing oxidative stress and consequently affecting activities of anti-oxidant enzymes.

The significant decrease in the activities of serum antioxidant enzymes, observed in the smoker-AGA group further confirms that AGA in smokers may be oxidative stress-induced, although activities of antioxidant enzymes was significantly reduced in alcohol group, but there was no correlation between AGA and duration of alcohol consumption. This relationship between smoking and oxidative stress, according to Trüeb (2003) may be the reason why tobacco smoking is the single most preventable cause of significant morbidity and an important cause of death in the general population, its adverse effects on the skin in which smoking induce premature skin (scalp) ageing may be a cause of the correlation between smoking and AGA observed in this study in which a higher percentage of men exhibiting AGA Norwood classification of grade IV and above are found among smokers than in those in alcohol group.

A lack of correlation between duration of alcohol consumption and AGA, observed through this study is in agreement with that of Severi et al. (2003), who also did not observe an association between alcohol consumption and AGA. In addition, a study has identified that more than 60% of those with early-onset AGA who were below the age of 30 years had either maternal grandfather or father who manifested severe AGA of Norwood classification of grade V or above which confirms the role of heredity in the manifestation of AGA.

## CONCLUSION

Although the findings of this study suggest a possible relationship between smoking and alopecia in the male Nigerian alopecia subjects, because of the positive correlation between duration of smoking and duration of androgenetic alopecia and because many of the subjects in AGA-smoker group presented with Norwood AGA classification of IV and above and were early onset in nature, still there is need for a more elaborate study, probably population-based one which will involve a large sample size and in which a more stringent criterion is employed to confirm if an association actually exist. This may probably provide an opportunity to increase public awareness of the danger of smoking especially as it relates to hair loss as well as offers an opportunity for general health education against smoking.

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