INTRODUCTION

Hair is a marvelous structure with cosmetic function. Androgenetic alopecia (AGA) is the most common hair loss condition affects both sexes; AGA is thinning of scalp hairs that initiated by androgens in susceptible patients.[1] The onset of AGA mostly in late adolescence, gradual and slowly develops over years. The frequency and the grade of severity of AGA increases with age.[2] Up to 30% of males will have AGA by 30 years, 50% by 50 years, and 80% by 70 years.[3]

Chitinase-3-like protein1 YKL-40 is a 40 kDa heparin and chitin-binding glycoprotein that has three N terminal amino acids: tyrosine (Y), lysine (K), and leucine (L).[4] It is produced by inflammatory cells as neutrophils, endothelial cells, macrophages, fibroblasts, chondrocytes and cells of smooth muscle.[5] Although YKL-40 biological function is unknown, YKL-40 participation has been also evidenced during extracellular matrix remodeling, migration and proliferation of (malignant) cells, angiogenesis, macrophage-induced inflammation, and T-cell activity.[6] Elevated YKL-40 are seen in diseases that characterized by active inflammation and remodeling as ischemic...
cardiovascular diseases (CVDs), atherosclerosis, diabetes mellitus (DM), metabolic syndrome (MS), inflammatory bowel disease, pneumonia, and cancer.[7]

YKL-40 would be a better biomarker for significant activity rather than perception of symptoms.[8] Several studies have investigated the role of YKL-40 in relation to cancer. YKL-40 levels are particularly high in recurrent cancer and highly differentiated cancers, which are characterized by high vascularization and a high turnover of extracellular matrix.[9]

The link between early AGA and associated of MS had been suggested.[10] AGA might be the indicator for arterial stiffness.[11] The link between YKL-40, MS, obesity, morbid obesity, and CVD is very complex; YKL-40 is strongly associated with 34% increase in serum level of triglycerides (TG) and twice increased of ischemic stroke attacks risk.[12] Serum YKL-40 level was significantly associated with the presence of MS. Serum YKL-40 may be a novel and useful indicator for MS.[13]

The serum level of YKL-40 and AGA may be linked by direct mechanism or through MS. Therefore, we aimed to evaluate the serum YKL-40 levels in patients with early and late onset AGA and as a sensitive biomarker for early AGA and for the detection of early hidden MS.

**MATERIALS AND METHODS**

**Study population**

This case–control study with total number of 100 individuals were enrolled; 70 AGA patients (equal number of males and females) and 30 apparently healthy controls. This study was approved by faculty of medicine related local ethics committee on research of humans and we obtained an informed consent from each subject prior the participation. This protocol of research work was in accordance with Helsinki declaration of the human rights.

Individuals with known history of CVDs, infections, cancer or any chronic inflammatory conditions hepatic, renal disorders, connective tissue disorders, or thyroid disorders that influence YKL-40 metabolism were excluded from the study.

AGA diagnosis was established by detailed medical history, clinical examination, and trichoscopic features using DermLite® 3 with 20× magnification power to assess hair density (hair/cm²) where number of hairs were assessed manually (more than 20% variability in hair diameter between affected and uninvolved areas). Especially females to exclude diffuse alopecia areata and frontal fibrosing alopecia, and onset was evaluated as early onset alopecia (by the age of 30 years or earlier)[14] and the various grades of AGA severity according to the Hamilton–Norwood classification in males and according to Ludwig classification in females. The diagnosis of MS was in accordance to criteria declared by National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII, 2001).[15] This was modified to add the World Health Organization’s waist circumference cutoff points, by the presence of 3 of the following: Abdominal circumference >102 cm in men and 88 cm in women. Hypertriglyceridemia >150 mg/dl. High-density lipoprotein cholesterol (HDL-C) <40 mg/dl in men and <50 mg/dl in women. Blood pressure (BP) >130/85 mmHg. Glycemia >100 mg/dl. BMI ≥25 was taken as overweight and ≥30 as obesity, per the WHO definition.[16]

**Laboratory tests**

A 5-mL of venous blood was obtained from each subject. Samples were centrifugated at 1262 g for about 10–15 min, aliquot and rapidly refrigerated at −20°C till the test. The serum level of YKL-40 was measured by a double antibody ELISA Kit from Sun Red Biotechnology Company, made in Shanghai, China, catalogue NO (201-12-2064).

**Statistical analysis**

Obtained data were statistically analyzed by SPSS (Statistical Package for Social Science) version 18 software (SPSS Inc., Chicago, IL, USA). The collected data were arranged as number (percentage) and were analyzed using Chi-square test. Mann–Whitney test for normally and non-normally distributed numerical variables, respectively. Pearson correlation coefficient also calculated the correlation between quantitative data.

Receiver operating characteristic (ROC) curve analysis was used to identify optimal cutoff values of different parameters with maximum sensitivity and specificity for prediction of the outcome. Accuracy is measured by the area under the ROC curve. An area of 1 represents a perfect test; an area of 0.5 represents a worthless test. A rough guide for classifying the accuracy of a diagnostic test is the traditional academic point system: 0.90–1 = excellent (A); 0.80–0.90 = good (B); 0.70–0.80 = fair (C); 0.60–0.70 = poor (D); and 0.50–0.60 = fail (F). The level of statistical significance in this work was $P \leq 0.05$.  

50  
International Journal of Trichology / Volume 12 / Issue 2 / March-April 2020
RESULTS

A total of 70 AGA patients, the range of duration was from 1 to 22 years and mean about 7.17 years, the mean age of patients was 38.5 ± 8.67 (ranging 18–50) years. Among enrolled patients; 51.4% had early onset AGA (by age 30 years or earlier) and 48.6% had late onset. The most common grades among male were II and VI (22.9% and 20%, respectively) and among female II and III (40% and 40%, respectively).

The mean serum levels of YKL-40 in AGA cases and control were (58.1 ± 72) ng/ml versus (11.8 ± 2.47) ng/ml. Patients showed highly significant higher serum YKL-40 level more than that of the healthy controls (P < 0.001) [Table 1]. There was highly significant increase in YKL-40 level among early onset male and female cases compared to late onset cases (99.06 ± 80.58), (97.13 ± 86.67) versus (16.02 ± 6.66), (15.49B ± 4.90) ng/ml, respectively (P < 0.001 each) [Table 2 and Figures 1, 2].

There was significant increase in MS association in AGA cases than controls (P < 0.05), and highly significant increase in MS associations and severity among early onset male and female cases compared to late onset cases (P < 0.001 each) [Table 3].

The mean serum levels of YKL-40 in AGA cases with MS were highly significantly higher (103.80 ± 86.31) ng/ml versus (19.62 ± 10.10) ng/ml those cases without (P < 0.001) [Table 4]. There was highly significant increase in YKL-40 level among early onset AGA with MS compared to late onset AGA with MS (P < 0.001 each).

There were significantly increased BP, total cholesterol (TC), TG, and fasting blood sugar (FBS) in all

Table 1: Comparison between cases and control groups in YKL-40 level

| Variable | YKL-40 | AGA (n=70) | Control (n=30) | MW test | P       |
|----------|--------|------------|----------------|---------|---------|
| X±SD     | 58.1±72| 11.8±2.47  | 4.78           | <0.001**|
| Median   | 23.05  | 11.9       |                |         |
| Range    | 1.4-290.7 | 7.1-15.5 |                |         |

**HS – Highly significant; AGA – Androgenetic alopecia; SD – Standard deviation; MW – Mann-Whitney test

Table 2: Comparison between early and late onset male and female androgenetic alopecia cases in YKL-40 level

| Variable | Early AGA | Late AGA | MW test | P       |
|----------|-----------|----------|---------|---------|
| Male cases (YKL-40) | n=18 | n=17 | 4.85 | <0.001**|
| X±SD     | 99.06±80.58 | 16.02±6.66 | (HS) |         |
| Median   | 41.75 | 16.8     |         |         |
| Range    | 20.40-290.70 | 8.6-26.6 |         |         |
| Female cases (YKL-40) | n=18 | n=17 | 4.72 | <0.001**|
| X±SD     | 97.13±86.67 | 15.49±4.90 | (HS) |         |
| Median   | 39.7 | 13.2     |         |         |
| Range    | 20.40-290.70 | 8.6-26.6 |         |         |

**HS – Highly significant; SD – Standard deviation; MW – Mann-Whitney test; AGA – Androgenetic alopecia

Table 3: Comparison between early and late onset male and female androgenetic alopecia cases as regards metabolic syndrome

| Variable | Early AGA | Late AGA | \( \chi^2 \) | P       |
|----------|-----------|----------|---------------|---------|
| Male cases | n=18 | n=17 | 13.05 | <0.001**|
| No MS    | 5 (27.8) | 15 (88.2) |               |         |
| Yes      | 13 (72.2) | 2 (11.8)  |               |         |
| Female cases | n=18 | n=17 | 24.12 | <0.001**|
| No MS    | 2 (11.1) | 16 (94.4) |               |         |
| Yes      | 16 (88.9) | 1 (5.9)   |               |         |

**HS – Highly significant; MS – Metabolic syndrome; AGA – Androgenetic alopecia

![Figure 1: Comparison between early and late cases in male group in YKL-40](image1)

![Figure 2: Comparison between early and late cases in female group in YKL-40](image2)
cases \((P < 0.05 \text{ each})\) except TG in early onset male cases was highly significantly increased \((P < 0.001)\) and significant decrease in HDL among early onset male and female cases \((P < 0.05)\).

Receiver operating curve analysis showed the sensitivity of serum YKL-40 in diagnosis of AGA at cutoff 14.25 was 81.4\%, specificity was 93.3\% and the accuracy was 83.5\% \((P < 0.001)\) and in diagnosis of early onset AGA at cutoff 20.35 was 97.2\%, specificity was 85.3\% and the accuracy was 91.4\% \((P < 0.001)\) [Figures 3 and 4].

**DISCUSSION**

AGA is a genetically determined disease with progressive course through its gradual conversion of hairs from terminal into vellus like hairs.\(^{[17]}\) Pathophysiology that links AGA and MS has not been fully established; excess androgens underpin both mechanisms.\(^{[18]}\)

As regards relation between AGA and YKL 40, we found that AGA patients had significant higher serum YKL-40 level more than control group \((P < 0.001)\). Furthermore, there was a highly significant increase in YKL-40 among early onset male and female cases compared to late onset cases \((P < 0.001 \text{ each})\) suggesting the possible role of YKL in AGA pathogenesis even in early stages, which can be explained by many mechanisms;

Cytokines, such as Transforming Growth Factor beta 1 (TGF-\(\beta 1\)), interleukin (IL)-1\(\alpha\), and Tumor Necrosis Factor alpha (TNF-\(\alpha\)), have inhibitory and pro-apoptotic effects that induce catagen.\(^{[19–21]}\) YKL-40 is stimulated by locally pro-inflammatory cytokines such as TNF-\(\alpha\) and IL-1\(\beta\).\(^{[14]}\) YKL-40 levels correlated with pro-inflammatory TNF\(\alpha\) and IL-1\(\beta\) levels.\(^{[22]}\)

Hair follicle micro-inflammation and AGA is a multistep process that could be involved in the generation of the inflammatory response.\(^{[23]}\) Langerhans cells or alternatively keratinocytes could present antigen to infiltrating T lymphocytes and induce T-cell proliferation. The antigens are selectively destroyed by infiltrating macrophages, or natural killer cells.\(^{[24]}\) On sustained inflammation, together with connective tissue remodeling, where collagenases play an active role. Collagenases are contributed to perifollicular fibrosis by preparing tissue matrix and basal membranes for macrophages and T-cell adhesion.\(^{[19]}\) Dermal papillae cells proliferation and differentiation were studied for their role in the pathogenesis of AGA as DPCs from balding scalp contain more androgen receptors than nonbalding scalp,\(^{[25]}\) and oxidative stress role in AGA pathogenesis by significant alteration of DPCs morphology, migration, proliferation, senescence, and TGF-\(\beta\) signaling.\(^{[26]}\) Whereas YKL-40 play a major role in epithelial-mesenchymal (transition, migration and proliferation), tissue differentiation, angiogenesis, remodeling and inflammation.\(^{[27]}\) YKL-40 produced by activated macrophages can promote Th1 immunity.\(^{[28]}\)

| Variable | YKL-40 | Cases with MS \((n=32)\) | Without MS \((n=38)\) | MW test | \(P\) |
|----------|--------|--------------------------|------------------------|---------|------|
| X±SD     | 103.8±86.31 | 19.6±10.10 | 4.95 | <0.001** |
| Median   | 90.2   | 15                       | (HS)                   |         |      |
| Range    | 12.9–290.7 | 1.4–50.2     |                        |         |      |

**Table 4: Relation between cases with and without metabolic syndrome in YKL-40 level**

**Figure 3:** Validity of YKL-40 in diagnosis of AGA

**Figure 4:** Validity of YKL-40 in diagnosis of early onset AGA among the studied group
Insulin resistance (IR) means at normal insulin level, target tissues are unable to mount normal glucose-lowering response by suppression of endogenous glucose production, suppression of lipolysis, cellular uptake of available plasma glucose, and net glycogen synthesis. This IR necessitates increased insulin secretion to compensate, so fasting plasma insulin levels increase.\(^{29}\) IR has a pathogenic role in the miniaturization of hair follicles. Vasoactive substances with endothelial dysfunction in IR lead to microcirculatory disturbance, perifollicular vasoconstriction, and vascular wall proliferation of smooth muscle. This leads to microvascular insufficiency, local-tissue hypoxia, and progressive miniaturization of hair follicles.\(^{30}\) High scores of IR showed in males with early onset AGA.\(^{31}\) YKL-40 was positively correlated with IR.\(^{32}\) YKL-40 was inversely correlated with insulin-like growth factor 1.\(^{33}\)

In this work, there was significant increase in MS in AGA cases than controls (\(P < 0.05\)), there was highly significant increase in MS associations among early onset male and female cases compared to late onset cases (\(P < 0.001\) each).

The relationship between AGA and MS is still matter of controversy. Studies have reported link between AGA and many chronic diseases separately but making elements of MS including hypertension, abnormal serum lipid profiles, obesity, IR, and CVD. However, other studies not supported these associations.

There was a significant association between AGA and MS as HDL-C was found to be of particular importance in patients with moderate or severe AGA.\(^{40}\) This was in line with Dharam Kumar study\(^{41}\) study who found a significant link between MS and AGA after adjusting other factors. In between components of MS, HDL is a specifically significant factor associated with AGA. Patients with moderate or severe AGA may have higher risk for developing MS and early detection of this in patients with moderate or severe AGA may be essential in early intervention to reduce the risk of dangerous complications. Similar results were seen in studies conducted by.\(^{10,42,43}\) In contrast to our study results reported by Mumcuoglu et al.’s study\(^{31}\) there were no significant difference between AGA patients and controls. As regards the onset, hypertension was highly prevalent in early-onset females with AGA that gives a clue for early-onset AGA to be a strong suggesting factor for early-onset severe coronary heart disease.\(^{44}\) Hormonal and metabolic abnormalities have been reported in men with early-onset AGA.\(^{45}\)

In this work, AGA patients with MS showed highly significant higher serum YKL-40 level more than that without (\(P < 0.001\)). There was highly significant increase in YKL-40 level among early onset AGA with MS compared to late onset cases with MS (\(P < 0.001\) each).

As regards higher YKL-40 level in AGA with MS than without can be explained as MS defined as a collection of many clinical signs mainly cardiovascular and diabetes\(^{46}\) and YKL-40 is strongly linked to same elements as MS, morbid obesity,\(^{46}\) DM type 2 and type 1 also.\(^{48}\)

There were significantly increased SBP, DBP, TC, TG, and FBS in all cases (\(P < 0.05\) each) Except TG in early onset male cases was highly significantly increased (\(P < 0.001\)) and significant decrease in HDL among early onset male and female cases (\(P < 0.05\)).

In males, the value of high-density lipoprotein in cases (48.43%) was lower and statistically very highly significant when compared to the controls. There was statistical significance increase in frequency of DM, HPT, and dyslipidemia among early onset AGA male cases.

**CONCLUSIONS**

This work had found that YKL-40 may have a role in the pathogenesis of AGA by direct mechanisms and indirectly through associated MS. We have also demonstrated that not only serum level of YKL 40 is increased in AGA patients but also that its level may reflects the early onset, long duration, and association with MS components and their severity. YKL-40 is a promising sensitive biomarker for understanding the pathogenesis of AGA and detection of early cases. AGA may be seen in older ages due to many factors in pathogenesis, however in our study, we found increased YKL 40 and MS components and severity in younger AGA persons with short-time disease duration, these data may increase awareness of doctors about susceptible patients as minimal lifestyle alterations in young early AGA patients can decrease the risk of MS.

These results were based on a relatively small number of patients that was a major limitation of this study and we recommend doing the work in larger scale of patient.

**Acknowledgments**

We are very grateful to all volunteers who took part in this study and the research team who collected the data.

**Financial support and sponsorship**

This was an authors’ own work. Laboratory investigations were done in clinical pathology laboratory.
Elhabak and Abdel Halim: YKL-40 a new sensitive predictor link for common association

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Peytavi UB, Blumeyer A, Tosti A, Finner A, Marmol V, Trakatelli M, et al. S1 guideline for diagnostic evaluation in androgenetic alopecia in men, women and adolescents. Br J Dermatol 2011;164:5-15.
2. Krupa Shankar D, Chakzavarti M, Shilpakar R. Male androgenetic alopecia: Population-based study in 1,005 subjects. Int J Trichology 2009;1:31-3.
3. Yip L, Zaloumis S, Irwin D, Selter G, Hopper J, Giles G, et al. Gene-wide association study between the aromatase gene (CYP19A1) and female pattern hair loss. Br J Dermatol 2009;161:289-29.
4. Umamathy D, Dormadula S, Krishnamoorthy E, Mariapanadar V, Viswanathavan V, Ramkumar KM. YKL-40: A biomarker for early nephropathy in type 2 diabetic patients and its association with inflammatory cytokines. Immunobiology 2018;223:718-27.
5. Johansen JS, Jensen BV, Roslund A, Nielsen D, Price PA. Serum YKL-40, a new prognostic biomarker in cancer patients: Cancer Epidemiol Biomarkers Prev 2006;15:194-202.
6. Junker N, Johansen JS, Andersen CB, Kristiansen BE. Expression of YKL-40 by tumoral macrophages in human small cell lung cancer. Lung Cancer 2005;48:223-31.
7. Kastrup J, Johansen JS, Winkel P, Hansen JF, Hildebrandt P, Jensen GB, et al. High serum YKL-40 concentration is associated with cardiovascular and all-cause mortality in patients with stable coronary artery disease. Eur Heart J 2009;30:1066-72.
8. Hansen JW, Thomsen SF, Porsbjerg C, Rasmussen LM, Harmesen L, Johansen JS, et al. YKL-40 and genetic status of CHI3L1 in a large group of asthmatics. Eur Clin Respir J 2015;2.5117.
9. Roslund A, Knopp AS, Jensen MB, Nielsen DL, Balslev E. YKL-40 protein expression is not a prognostic marker in patients with primary breast cancer. Breast Cancer Res Treat 2008;112:275-85.
10. Acibicu F, Kayatas M, Candan F. The association of insulin resistance and metabolic syndrome in early androgenetic alopecia. Singapore Med J 2010;51:931-6.
11. Agac MT, Bektas H, Korkmaz L, Erkan H, Gurbak I, Acibucu F, Kayatas M, Candan F. The association of insulin resistance and metabolic syndrome in early androgenetic alopecia and metabolic syndrome in a young Indian population. Int J Trichology 2009;1:131-3.
12. Biyema N, Kucerova R, Fiuzechova M, Hajduch M, Kolaz Z. Androgenetic alopecia and current methods of treatment. Acta Dermatovenerol Alp Pannonica Adriat 2005;1:4-5-8.
13. Jefri M, Huang YN, Huang WC, Tai CS, Chen WL. YKL-40 regulated epithelial-mesenchymal transition and migration/invasion enhancement in non-small cell lung cancer. BMC Cancer 2015;15:590.
14. Di Rosa M, Malaguarnera G, De Gregorio C, Drago F, Malaguarnera L. Evaluation of CHI3L-1 and CHIT-1 expression in differentiated and polarized macrophages. Inflammation 2013;36:482-92.
15. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. Physiol Rev 2018;98:2133-223.
16. Matilainen V, Laakso M, Hirssro P, Koskela P, Rajala U, Keinäinen-Kiukkami S. Hair loss, insulin resistance, and heredity in middle-aged women. A population-based study. J Cardiovasc Risk 2003;10:227-31.
17. Muncucuoglu C, Elmekei TR, Ucak S. The investigation of insulin resistance and metabolic syndrome in male patients with early-onset androgenetic alopecia. Eur J Dermatol 2010;20:79-82.
18. Andreasen M, Raymond I, Hildebrandt P, Kistorp C, Rathcke C, Vestergaard H. Associations between plasma insulin-like growth factor-I and the markers of inflammation interleukin 6, C-reactive protein and YKL-40 in an elderly background population. Inflamm Res 2010;59:503-10.
19. Ahousou S, Le Toumelin P, Crickx B, Descamps V. Association of androgenetic alopecia and hypertension. Eur J Dermatol 2007;17:220-2.
20. Matilainen V, Koskela P, Keinäinen-Kiukkaami S. Early androgenetic alopecia as a marker of insulin resistance. Lancet 2000;356:1165-6.
21. Sadighia A, Zadeh GM. Evaluation of lipid levels in androgenetic alopecia in comparison with control group. J Eur Acad Dermatol Venereol 2009;23:80-1.
22. González-González JG, Mancillas-Adame LG, Fernández-Reyes M, Gómez-Flores M, Lavalle-González FJ, Ocampo-Candiani J, et al. Androgenetic alopecia and insulin resistance in young men. Clin Endocrinol (Oxf) 2009;71:494-9.
23. Matilainen VA, Mikkonen PK, Keinäinen-Kiukkami SM. Early onset of androgenetic alopecia associated with early severe coronary heart disease: A population-based, case-control study. J Cardiovasc Risk 2001;8:147-51.
24. Rebona A, Balhness and coronary artery disease: The dermatologic point of view of a controversial issue. Arch Dermatol 2001;137:943-7.
25. Dogramaci AC, Balei DD, Balei A, Karazincir S, Savas N, Topaloglu C, et al. Is androgenetic alopecia a risk for atherosclerosis? J Eur Acad Dermatol Venereol 2009;23:673-7.
26. Su LH, Chen TH. Association of androgenetic alopecia with metabolic syndrome in men: A community-based study. Br J Dermatol 2010;163:371-7.
27. Dhamar-Kumar KC, Kiman Kumar YH, Neladimmanahally V. Association of androgenetic alopecia with metabolic syndrome: A case-control study on 100 patients in a tertiary care hospital in South India. Indian J Endocrinol Metab 2018;22:196-9.
28. Chakrabarty S, Harhanar R, Goswad D, Suresh H. Association of premature androgenetic alopecia and metabolic syndrome in a young Indian population. Int J Trichol 2014;6:50-3.
Elhabak and Abdel Halim: YKL-40 a new sensitive predictor link for common association

43. Bakry OA, Shoeib MA, El Shafiee MK, Hassan A. Androgenetic alopecia, metabolic syndrome, and insulin resistance: Is there any association? A case-control study. Indian Dermatol Online J 2014;5:276-81.

44. Arias-Santiago S, Gutiérrez-Salmerón MT, Castellote-Caballero L, Buendía-Eisman A, Naranjo-Sintes R. Androgenetic alopecia and cardiovascular risk factors in men and women: A comparative study. J Am Acad Dermatol 2010;63:420-9.

45. Cannarella R, La Vignera S, Condorelli RA, Calogero AE. Glycolipid and hormonal profiles in young men with early-onset androgenetic alopecia: A meta-analysis. Sci Rep 2017;7:7801.

46. Hempen M, Kopp HP, Elhenicky M, Höbaus C, Brix JM, Koppensteiner R, et al. YKL-40 is elevated in morbidly obese patients and declines after weight loss. Obes Surg 2009;19:1557-63.

47. Brix JM, Höllerl F, Koppensteiner R, Schernthaner G, Schernthaner GH. Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. J Exp Med 2009;206:1149-66.

48. Thomsen SB, Gjesing AP, Rathcke CN, Ekstrom CT, Eiberg H, Hansen T, et al. Associations of the inflammatory marker YKL-40 with measures of obesity and dyslipidaemia in individuals at high risk of type 2 diabetes. PLoS One 2015;10:e0133672.