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ORIGINAL RESEARCH

Serum Insulin and Atherosclerotic Markers in Acanthosis Nigricans Patients

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ABSTRACT

Background: To study serum insulin levels and atherosclerotic markers in patients with acanthosis nigricans in a rural tertiary care center.

Materials and Methods: It was a Cross sectional study. The study was conducted in the Departments of Biochemistry and Dermatology, Venereology, Leprosy (DVL), at Kamineni Institute of Medical Sciences, India.

Results: Total number of patients recruited for the study was 50. Mean age of the patient was 26.8 years. Females (60%) have outnumbered males (40%). Overall majority of the acanthosis nigricans patients were students (46%). Among females majority of them were housewives (22%). In a majority of the patients, neck was the initial site of darkening (82%). Most of them have duration of darkening of skin for more than one year (64%) and 84% of the patients had an insidious onset. Forty six percent of patients had associated medical illness such as hypothyroidism (22%), diabetes mellitus (10%), hypertension (8%), polycystic ovarian syndrome (4%), psoriasis (2%). Neck(98%) is the most common site of acanthosis nigricans followed by axilla(88%); elbow(42%); periorbital region(36%); groin(34%); knees(24%); temples(22%); infralabial region(20%); antecubital fossa(14%); perioral and perinasal (12% each); knuckles(10%); skin over hyoid bone(8%); sides of waist and inframammary areas(6% each) and popliteal fossa(2%). Out of 50 patients, raised serum insulin levels seen in 54% patients, of which 42% are non-diabetics and 12% are diabetics. Also, 96% had normal fasting blood sugars (FBS <110mg/dL), 80% had normal postprandial blood sugars (PPBS <140mg/dL). All patients (100%) had normal total cholesterol (< 250mg/dL), 80% patients had normal HDLc (>35mg/dL), 98% had normal LDLc (< 130mg/dL), 80% had normal triglycerides (<160mg/dL), 86% had normal VLDLc < 40mg/dL, out of the total 50 patients with acanthosis nigricans.

Conclusion: Hypothyroidism was present in 22% of the patients with acanthosis nigricans whereas diabetes mellitus was present in 10% of the patients with acanthosis nigricans. Levels of fasting serum insulin were raised in 54% of patients having burke's neck severity score of 2, 3, 4 and neck texture score of 2,3 and axilla severity score of 2,3 in patients with acanthosis nigricans. About 20% of studied patients had decreased HDL cholesterol levels(<35 mg/dL and increased serum triglycerides(>160 mg/dL).

Keywords: Acanthosis nigricans; Fasting Insulin; Axilla; Triglycerides; Neck severity; Popliteal fossa; Hypothyroidism.

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INTRODUCTION

Acanthosis nigricans is a velvety, darkening of the skin that usually occurs in intertriginous areas. This hyperpigmentation has poorly defined borders, usually occurs in skin fold areas, such as the back of the neck, axilla, and groin, antecubital and popliteal fossae, umbilical, perianal areas, and in advanced conditions, dorsum of hands and fingers12 and may include thickening of the skin.[1]

Acanthosis nigricans is most commonly associated with diabetes, insulin resistance (IR), and obesity,[2] but rarely it can be a sign of internal malignancy. It can also occur with hormone disorders, and with the use of certain medications like systemic glucocorticoids and oral contraceptives. Early detection of IR may allow us time to intervene well before the development of DM Type-2. Bhagyanathan M et al, in their study, showed that 62% of children having AN had high IR.[3]

Some studies had reported the different prevalence of the AN in different regions of the world, for example, one study had reported 7% in unselected populations in Galveston, Texas, the United States and another study had reported 74% in obese people in Dallas, Texas, the United States. The prevalence of AN in New Mexico was 49.2% in obese adolescents compared with 7.7% in those who were not obese.[4]

AN is an important predictor of the insulin resistance in childhood obesity.13 Cardiovascular disease (CVD) that is associated with the metabolic syndrome may be explained by the presence of insulin resistance (IR) and there is compelling, long-standing evidence that both high triglycerides and a low plasma HDLc are a frequent consequence of IR.[5]

Sustained efforts to attain and maintain a healthy weight through improved dietary intake and increased physical activity can reduce insulin resistance and prevent associated complications.[6]

Aim of the study:

To study serum insulin and atherosclerotic lipid profile in acanthosis nigricans patients of a rural tertiary care center.

Objectives of the study:

To evaluate the morphological patterns of acanthosis nigricans.

To evaluate hyperinsulinemia and its association with severity of acanthosis nigricans.

To study the percentage of diabetes mellitus in patients with acanthosis nigricans.

MATERIALS & METHODS

A cross sectional study has been conducted after approval from Institutional Ethics Committee in the Department of Biochemistry and Dermatology, Venereology and Leprosy at Kamineni Institute of Medical Sciences, Narketpally. The study duration was 2 years from October 2018 to September 2020 and a total of 50 patients were included in this study.

Patients of all age groups diagnosed clinically with acanthosis nigricans were included in the study and those patients not willing for study, patients with acute illness, Pregnant and lactating women and patients on medications which can cause drug induced acanthosis nigricans were excluded from the study.

A detailed history of all patients based on standard questionnaire has been taken with emphasis on history of Darkening of skin, Duration, Onset (acute: <2 months, chronic: >2 months), and Initial site and distribution.

Dermatological Examination:-
Acanthosis nigricans is mostly diagnosed clinically by its morphological appearance. Sites examined in the patients are neck (back,sides,front), axilla(Rt/Lt), elbow(Rt/Lt), antecubital fossa(Rt/Lt), popliteal fossa(Rt/Lt), knuckles, temples, around eyes, nasolabial fold, infralabial region, periumbilical region, groin(Rt/Lt), knee(Rt/Lt), oral cavity, tongue, palms(Rt/Lt). Acanthosis nigricans severity over neck and axilla has been scored according to Burke's quantitative scale. Acanthosis nigricans texture over neck was also scored. Systemic examination has been done.

Investigations Performed:
Venous blood samples were collected in fasting state from patients and analyzed for serum insulin level (by ELISA method, DIAsource Immuno Assay SA), Fasting blood sugars, (FBS) and Fasting lipid profile which includes total cholesterol, triglycerides, LDLc, VLDLc, HDLc were estimated using clinical chemistry autoanalyzer. Post prandial venous blood sample collected for estimation of post prandial blood sugar levels.

Statistical Analysis
Statistical software SPSS 19.0 has been used for analyzing the data (mean and standard deviation). Paired t-test has been used for statistical significance for hirsutism whereas student t-test (two tailed, independent) was used for calculating the statistical significance of other parameters. A p value of <0.05 considered statistically significant.

RESULTS
Out of 50 patients studied for Acanthosis nigricans, females (60% N=30) have outnumbered males (40% N=20) and 44% of patients were in age group of 21-30years, 28% were in age group of 11-20years, 14% were in age group of 31-40years, 10% were in age group of >40years, 2% were in the age group 1-10years, 2% were in the age group >50 years. Maximum number of patients was seen between age group of 21-30 years [Table1].

Table 1: Agewise distribution of patients with acanthosis nigricans

Age in years	Number of patients(n=50)	Percentage (%)
1-10	1	2
11-20	14	28
21-30	22	44
31-40	7	14
41-50	5	10
>50	1	2
Total	50	100

Most of the patients N=32 (64%) had duration of darkening of skin for more than 1year. Most of them (84%) had an insidious onset. Neck was the most common initial site of darkening (82%), followed by axilla (12%), face (2%), perioral (2%) and temple area (2%) [Table2].
Twenty three out of 50 studied patients (46%) had associated medical illness such as hypothyroidism (22%), diabetes mellitus (10%), hypertension(8%), polycystic ovarian syndrome (4%), psoriasis(2%) and 36% had a family history of diabetes mellitus, 6% had a positive family history of acanthosis nigricans, 4% had a family history of hypertension and hypothyroidism respectively [Table3].
Neck (98%) is the most common site of acanthosis nigricans followed by axilla(88%); elbow(42%); periorbital region(36%); groin(34%); knees(24%); temples(22%); infralabial region(20%); antecubital fossa(14%); perioral and perinasal (12% each); knuckles(10%); skin

over hyoid bone(8%); sides of waist and inframammary areas(6% each) and popliteal fossa(2%) [Table4].

Table 2: Distribution of patients based on history related to darkening of skin with duration, onset and initial site of darkening.

Parameters		**Number of patients (N=50)**	**Percentage (%)**
Duration of darkening ofskin	<or=1 year	18	36
	>1year	32	64
	Total	50	100
Onset of darkening ofskin	Acute	8	16
	Insidious	42	84
	Total	50	100
Initialsiteofdarkening	Neck	41	82
	Axilla	6	12
	Face	1	2
	Perioral	1	2
	Temple	1	2
	Total	50	100

Table 3: Distribution of patients based on history of medical illness

History of medical illness	**Number of patients studied (N=50)**	**Percentage (%)**
No	27	54
Yes	23	46
Diabetes mellitus(DM)	5	10
Hypertension(HTN)	4	8
PCOS	2	4
Hypothyroidism	11	22
Psoriasis	1	2
Total	50	100

Table 4: Distribution of patients based on sites having acanthosis nigricans on examination

Other sites on examination	**Number of patients**	**Percentage (%)**
Periorbital	18	36
Temples	11	22
Perinasal	6	12
Perioral	6	12
Infralabial	10	20
Skinoverhyoidbone	4	8
Neck		98
Axilla	44	88
Antecubitalfossa	7	14
Elbow	21	42
Knuckles	5	10
Sides of waist	3	6
Inframammary	3	6
Periumbilical	0	0
Groin	17	34
Popliteal fossa	1	2

Knees	12	24
Palms	0	0

Table 5: Distribution of patients based on neck severity score and the association of neck severity with serum insulin levels.

Burke's Neck Severity Score	Number of patients with percentage (N=50)	Serum insulin(µ IU/mL)	
		Normal (N=23)	Raised (N=27)
Score 0	1(2%)	0(0%)	1(3.7%)
Score 1	8(16%)	2(8.7%)	6(22.2%)
Score 2	11(22%)	6(26.1%)	5(18.5%)
Score 3	16(32%)	10(43.5%)	6(22.2%)
Score 4	14(28%)	5(21.7%)	9(33.4%)

Out of total patients, 2% had score 0, 16% had score 1, 22% had score 2, 32% had score 3 and 28% had score 4 neck severity of acanthosis nigricans. Out of the 27 patients with increased serum insulin levels, the neck severity score of 4 seen in 33.4% of patients and that of 1 and 3 score seen in 22.2% patients respectively. Neck severity score of 2 seen in 18.5% of Acanthosis nigricans patients with raised insulin levels [Table5].

Out of the total 50 patients with Acanthosis nigricans, 27(54%) patients had raised serum insulin levels, of which 21(42%) are non-diabetics and 6(12%) are diabetics. Forty-eight (96%) had FBS <110mg/dL, 2(4%) had FBS >110mg/dL. Forty patients (80%) had PPBS <140mg/dL and 10(20%) had PPBS >140mg/dL. All the studied patients (100%) had total cholesterol <250mg/dL and none had total cholesterol >250mg/dL. Ten (20%) had HDLc <35mg/dL and 40(80%) had HDLc >35mg/dL. Forty-nine (98%) had LDLc <130mg/dL and 1(2%) had LDLc >130mg/dL. Forty (80%) had triglycerides <160mg/dL and 10(20%) had triglycerides >160mg/dL. Forty-three (86%) had VLDLc <40mg/dL and 7(14%) had VLDLc >40mg/dL.

Table 6: Comparison of clinical variables of patients with serum insulin levels

Clinical variables	Serum Insulin (µIU/mL)		P value
	Normal Insulin levels (N=23)	Raised Insulin levels (N=27)	
Serum Insulin (µIU/mL) (mean±SD)	9.2±5.6	31.3±16.8	0.001
Age(years)(mean±SD)	26.35 ±9.8	27.26±11.6	0.765
Gender(male: female)	10:13	10:17	0.643
BMI(mean±SD)	26.98±4.8	26.29±5	0.629
Waist-hipratio(mean±SD)	0.93± 0.9	0.06±0.07	0.496
Total cholesterol (mg/dL) (mean±SD)	167.65±26.7	165.44±28.1	0.777
Triglycerides (mg/dL)(mean±SD)	131.30±44.3	145.11±83.5	0.461
LDLc (mg/dL) (mean±SD)	89.64±13.6	89.95±15.1	0.940
VLDLc (mg/dL) (mean±SD)	27.89±9.8	29.84±9.8	0.488
HDLc (mg/dL) (mean±SD)	44.70±13.7	42.50±7.6	0.501
FBS (mg/dL) (mean±SD)	84.17±10.0	88.56±15.9	0.243
PPBS (mg/dL) (mean±SD)	122.43±18.6	119.04±31.4	0.639

Raised serum insulin levels 31.3±16.8µIU/mL seen in 27 patients (54%) is more positively correlated in acanthosis nigricans patients and statistically significant with P <0.001 [Table6]. The normal levels of insulin 9.2±5.6µIU/mL was observed in 23 patients (46%). All the

above clinical variables were statistically not significant with raised serum insulin levels in studied patients [Table6].

DISCUSSION

Acanthosis nigricans is a dermatosis characterized by velvety, papillomatous, brownish-black, hyperkeratotic plaques, typically on the intertriginous surfaces and neck. It can be a manifestation of systemic disease that is associated with insulin resistance, diabetes mellitus, obesity, internal malignancy, endocrine disorders, and drugs. It is a marker of insulin resistance and hyperinsulinemia, with or without diabetes mellitus. Therefore this study has been done to correlate serum insulin levels with acanthosis nigricans and its severity. Results have been compared with few related studies published in Indian and worldwide literature. In the present study, 50 cases of acanthosis nigricans were studied to correlate the serum insulin levels with acanthosis nigricans. Majority of the patients were between 21- 30 years. Mean age ± standard deviation= 26.8 ± 10.69. Mean age in this study was 26.8 which was comparable to the studies done by Shah NG et al 2019 (32.47).[7]

In this study, females (60%) have outnumbered males (40%) (male: female= 1:1.5). This is comparable to studies carried out by Shah NG 2019 (females - 62% and males -38%),7 Nithun TM et al 2019 (females-75% and males- 25%).[8]

Aanthosis nigricans over neck in the present study (98%) was comparable with the studies done by Shah NG 2019 et al (100%), Nithun TM et al 2019 (90%), Grandhe et al (93.5%), Puri N et al 2010 (93.3%).[8-10]

Acanthosis nigricans over axilla in the present study (88%) was more than the studies done by Shah NG 2019 et al (31%) and comparable with Varthakavi PK 2002 et al (80.6%).[7,11]

The percentage of patients with acanthosis nigricans over inframammary region(6%) and groin(34%) is more than the study done by Shah NG et al 2019 (submammary area-1% and groins-9%),46 but less than the study done by Nithun TM et al 2019 (groin-58.3%),[8] Varthakavi PK 2002 et al (infra-mammary region-10.3% and groins-61.1%).[11]

Percentage of patients with acanthosis nigricans over flexures of groins, knees and elbow was not calculated individually and was 40% all together in the study done by Puri N et al 2010.[10] In the present study, elbows (42%), knees(24%) and groins(34%) calculated separately. Aacanthosis nigricans in the present study (antecubital fossa-14% and knuckles-10%) were higher than the study done by Shah NG et al 2019(antecubital fossa-4% and knuckles-3%).[7]

Other sites involved in this study in descending order were periorbital region(36%); temples(22%); infralabial region(20%); perioral and perinasal (12% each); skin over hyoid bone(8%); sides of waist (6%) and popliteal fossa(2%).

Scoring of acanthosis nigricans over the neck and axilla was done according to the Burke's quantitative scale. Acanthosis nigricans severity seen over the neck was scored as 0 to 4 and that of axilla was scored as 0 to 4. Acanthosis nigricans texture seen over neck was scored as 0 to 3. In the present study, neck severity is statistically not significant with raised serum insulin levels with P=0.292, whereas there was a statistically significant correlation of increasing neck severity with each component of metabolic syndrome in the study done by Shah NG et al 2019,[7] Score 2, score 3, score 4 were more positively associated with raised serum insulin levels. Venkatswami S et al 2014, reported grade 3 and 4 neck severity were more predictive of raised serum insulin levels (P≤0.001).[12] Abeer KM et al 2013, reported positive correlation between neck severity and fasting serum insulin levels (P≤0.001).[13] In the present study, neck texture is statistically not significant with raised serum insulin (P=0.776) which was not comparable to the study done by Choudary SV 2017 (P=0.010).

Also score 2 and score 3 were more positively associated with raised serum insulin levels which was comparable to the study done by Venkatswami S et al (neck texture 2 and 3 scores

were more suggestive of raised insulin levels with P=0.004).12 Axilla severity is statistically not significant with serum insulin levels with P=0.30.

In the present study, neck severity of score 3 seen in 16% and score 4 in 14% cases of acanthosis nigricans which is less compared to studies done by Nithun TM et al (score 3 - 30% and score 4 -20%).48 • In the present study, neck texture of score 3 seen in 46% cases which is more compared to study done by Nithun TM et al(23.3%).48 • Also, in the present study, axilla severity of score 2 in 42%, score 3 in 28%, score 4 in 14% cases which is less compared to study done by Nithun TM et al(score 4-20%).4 Scoring was done by Burke's quantitative scale,11,25 but results could not be compared with that of Burke JP et al, as in the present study, a single observer has performed the study but in the study done by Burke JP et al,11 a total of 406 subjects were independently examined for AN by at least two observers.

Relationship between acanthosis nigricans and serum insulin levels:

In the present study, fasting serum insulin levels were raised in 54% which is more compared to study done by Choudhary SV 2017(35.8%).25 Fasting serum insulin levels range is from 0.27(minimum) to 104.70(maximum). Mean insulin levels in the present study (21.62±17.27) which was comparable to study done by Nithun TM et al 2019 (19.3).48 The percentage of patients with acanthosis nigricans having raised fasting serum insulin in the present study (54%) was higher than the study done by Nithun TM (23.3%),8 Puri N (40%)10 and was lower than the study done by Varthakavi PK 2002 et al (100%).[11]

In the present study, raised serum insulin is more positively correlated in acanthosis nigricans patients and is statistically significant (P≤0.001) which was comparable to studies done by Choudhary SV 2017 (P≤0.001).[14]

Serum triglycerides were raised in 20% patients which was less compared to study done by Shah NG et al (61%).4 In the present study, HDL cholesterol were lower than normal levels in 20% patients which was less compared to study done by Shah NG et al (61%).[7]

In the present study, mean value of FBS (86.54) was less compared to the Mean value of PPBS in the present study (120.6) and the later was less compared to the study done by Varthakavi PK 2002 et al (186.9).[11]

CONCLUSION

Acanthosis nigricans is mostly seen in the age group of 21-30 years. It is predominantly seen in females, of which most of them were housewives. Most of the patients have neck (98%) as the commonest site of acanthosis nigricans followed by axilla (88%). Least commonly involved sites in the present study were popliteal fossa and infra-mammary areas.

Acanthosis nigricans patients with neck severity score 2, 3 and 4 (P=0.292) and neck texture score 2 and 3 (P=0.776) were more positively associated with raised serum insulin though statistically not significant. Axilla severity score was statistically not significant with serum insulin levels with P=0.30, but score 2 and score 3 were more positively associated with raised serum insulin levels. Hypothyroidism was present in 22% of the patients with acanthosis nigricans whereas diabetes mellitus was present in 10% of the patients with acanthosis nigricans. Levels of fasting serum insulin were raised in 54% of patients with acanthosis nigricans.

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