Assessment of Fasting Blood Glucose, HbA1c, Cholesterol and Triglycerides in Patient with Skin Tags in Khartoum State

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صدق الله العظيم

سورة الذاريات (آية 21)
This thesis is dedicated to: The sake of Allah, my Creator and my Master, My great teacher and messenger, Mohammed (May Allah blesses and grants him), who taught us the purpose of life.

I also dedicate my dissertation study to my family. A special feeling of gratitude to my loving parents, my husband Ahmed, my sons, Dr. Abdelwahab, My sisters and my brothers Who have never left my side and are very special.

To friends who encouraged and supported me, Heba, Walaa, Ebtihal, and all the people in my life who touched my heart, I dedicate this research.
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In the name of Allah, the Most Gracious and the Most Merciful (Alhamdulillah), all praises to Allah for the strengths and His blessing in completing this thesis.

At the end of this thesis I would like to thank all those people who made this thesis possible. At the end of this thesis, it is a pleasant task to express my thanks to all those who contributed in many ways to the success of this study and made it an unforgettable experience for me.

At this moment of accomplishment, first of all I pay homage to my guide,

Dr. Abdelwahab Abdien Saeed. This work would not have been possible without his guidance, support and encouragement. Under his guidance I successfully overcame many difficulties and learned a lot, he used to review my thesis progress, give his valuable suggestions and made corrections. His unflinching courage and conviction, will always inspire me. I can only say a proper thanks to him through my future work.

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Abstract

**Background:** Skin tags (acrochordons) are the common small benign connective tissue tumor of the dermis that occurs in old subjects usually located on the neck and the major flexors. And the aim of this study to estimate the prevalence of Diabetes Mellitus and dyslipidemia among patients with Skin Tags in Khartoum state.

**Materials and Methods:** This is descriptive case control study and was conducted in period from March To July 2018. In this study 50[28 (56%) male and 22(54%) female] patients with skin tags and 20[11(55%) male and 9(45%) female] healthy individual are randomly selected, blood samples were collected after fulfillment of questionnaire, fasting venous blood collected in fluoride oxalate container for fasting glucose, cholesterol and triglycerides and 2.5ml venous blood in EDTA for HbA1c, and were analysis by spectrophotometer for glucose cholesterol and triglycerides and ichroma for HbA1c, obtained results were analyzed statistically by using SPSS.

**Results:** The mean of fasting blood glucose, HbA1c, cholesterol and triglycerides of case is (111mg.dl, 5.6%, 205mg.dl, 160mg.dl) respectively. And the mean of fasting blood glucose, HbA1c, cholesterol and triglycerides of healthy individual is (86mg.dl, 5.0%, 149mg.dl, 170mg.dl) respectively. 62.2% of male has (1-10 ST), 38.5% of male has (11-20 ST) and 37.8% of female has (1-10 ST), 61.5% has 11-20 ST. case aged 20-40 years (48.6% has 1-10 ST and 15.4% has 11-20 ST), case aged 41-80 years (51.4% has 1-10 ST and 84.6% has 11-20 ST).

**Conclusion:** There was significant correlation between number of skin tags and mean levels of fasting blood glucose, HbA1c, and fasting serum cholesterol levels, no significant correlation between gender and number of ST, significant correlation between age and number of ST.
المستخلص

المقدمة: الزوائد الجلدية تعتبر من الأورام المديدة الشائعة. تحدث عند الكبار وعادة تكون في العنق ومعظم الأعضاء. هناك احتمال وجود علاقة بين هذه الزوائد والتبخير في استقلاب الكاريوبودرات (الجلوكوز) واستقلاب الدهون. الهدف من هذه الدراسة تقييم انتشار مرض السكري وارتفاع نسبة الدهون لدى المرضى الذين لديهم زوائد جلدية، في ولاية الخرطوم.

الطرق والادوات: هذه الدراسة دراسة مقارنة وصفية من مارس إلى يوليو 2018 تم اختيار 50 مريض بشكل عشوائي { 28 (56%) رجل و22 (45%) امرأة } و20 ليس لهم زوائد للمقارنة { 11 (55%) رجل و9 (45%) امرأة }. استخدمت العينات بعد ملء الاستمارات بين المشتركين في البحث وتم تحليل نسبة الجلوكوز والهيموغلوبين السكري والكوليسترول والدهون الثلاثية في الدم. وحللت النتائج باستخدام برامج الحاسوب.

النتائج: نسبة متوسط الجلوكوز والهيموغلوبين السكري والكوليسترول والدهون الثلاثية هي (111 و8,5 و20 و50 و160) على التوالي للمرضى الذين لديهم زوائد جلدية، ونسبة متوسط الجلوكوز والهيموغلوبين السكري والكوليسترول والدهون الثلاثية هي (86، 5، 149، 170) على التوالي للمشاركين الذين ليس لهم زوائد جلدية. 62.2% من الرجال لديهم (10-20 زوائد جلدية) 38.5% منهم لديهم (11-20 زوائد جلدية) و 37.8% من النساء لديهم (1-10 زوائد جلدية). 61.5% لديهم (11-20 زوائد جلدية). المشاركين من عمر 20 إلى 40 عام (48.6%) لديهم 1-10 زوائد جلدية و 15.4% لديهم 11-20 زوائد جلدية) والمشاركين من عمر 40 إلى 80 عام (51.4% لديهم 1-10 زوائد جلدية و 48.6% لديهم 11-20 زوائد جلدية).

الخلاصة: وجدنا في الدراسة أن هناك علاقة واضحة بين متوسط الجلوكوز والهيموغلوبين السكري والكوليسترول وبين عدد الزوائد الجلدية، وعلاقة طردية بين عدد الزوائد الجلدية والعمر، ولست هناك علاقة بين الجنس وعدد الزوائد الجلدية.
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<td>Deciliter</td>
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Chapter One
1.1 Introduction

Skin tags: Skin tags (acrochordons) are the common small benign connective tissue tumor of the dermis, most are minute 1 to 5 mm in the length, flesh colored to hyper pigmented pedunculated papilloma.\textsuperscript{(1)} Characteristically attached by short, thin stalk. They are most common on the neck, axilla and skin folds.\textsuperscript{(2)} They are also name soft fibromas, fibro epithelial polyps.\textsuperscript{(3)} These lesions are extremely common in adult population over 40 year of age and increase incidence in the elderly.\textsuperscript{(4)} Acrochordons are most frequent in obesity\textsuperscript{(5)}, hormonal imbalance\textsuperscript{(6)} metabolic syndrome\textsuperscript{(7)} and other condition have been reported as contributing factors. Histological, Skin tags classify as fibromas with hyperplasic epidermis connected to the skin on connective tissue stalk.\textsuperscript{(8)} The over lining epidermis is essentially normal. The skin tags appear as an outgrowth of skin. The dermis appear normal and there is a minimal inflammatory infiltrate present.\textsuperscript{(1)} Skin tags remain asymptomatic and are usually not painful unless they become inflamed or irritated.\textsuperscript{(9)} Most patients with skin tags consult a doctor for cosmetic reasons. Multiple STs are frequently associated with non- insulin dependent diabetes mellitus and obesity.\textsuperscript{(5)}

Clinical findings: Skin tags can be found throughout the adult population. They have no sex or race predilection. They are completely benign skin growth that has no malignant potential. Skin tags are almost never seen in children. Most skin tags are minute 1 to 5 mm in length, with a skin-colored to slightly hyper pigmented appearance.\textsuperscript{(10)} The lesion develop on the skin surface that rub together or that chronically rub against clothes.\textsuperscript{(11)}
**Histology:** Skin tag histological classifies as fibromas with hyperplastic epidermis connected to the skin on connective tissue stalk\(^{(11)}\). The overlying epidermis is essentially normal. The skin tag appears as an outgrowth of the skin. The dermis appears normal, and there is a minimal inflammatory infiltrate present.\(^{(10)}\)
1.2 Rationale

- Skin is the system in the body that can reflect many metabolic disorders; so can assist early diagnosis.
- Most Sudanese people don’t aware about skin tags because it is harmless and painless unless its inflamed or irritated, but others consider it as ugly singes and removed only for cosmetic purpose regardless of the causes of their appearance.
- This study can assist dermatologist to evaluate the patients with acrochordons for the presence of diabetes mellitus and dyslipidemas.
1.3 Objectives:

3.1.1 General objective:
To assess Fasting Blood Glucose (FBG), HbA1c, cholesterol and triglycerides among skin tags patient in Khartoum state.

3.1.2 Specific objectives:
- To measure FBG, HbA1c, cholesterol and triglycerides in case and control group.
- To compare between means of fasting blood glucose, HbA1c, serum cholesterol and serum triglycerides in patients with skin tags and in healthy individuals.
- To compare between the mean of FBS, HbA1c, S.Ch and TG in patients with different number of skin tags.
- To compare between the frequency and percentage of gender and number of skin tags.
- To compare between the frequency and percentage of age and number of skin tags.
Chapter Two
2. Literature Review

2.1 Skin manifestation of diabetes mellitus:

The human skin is the outer covering of the body. In humans, it is the largest organ of the integumentary system. The skin has multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments and internal organs.\(^{(12)}\)

Diabetes can affect every part of the body, including the skin. As many as 33 percent of people with diabetes will have a skin disorder caused or affected by diabetes at some time in their lives. In fact, such problems are sometimes the first sign that a person has diabetes. Luckily, most skin conditions can be prevented or easily treated if caught early.\(^{(13)}\)

Coetaneous manifestations in the setting of diabetes can be classified to non infectious, Infectious, Related to complication because of vasculopathy and Related to complication of diabetes treatment.\(^{(14)}\)

Insulin signaling supports normal skin proliferation, differentiation, and maintenance so in Diabetes mellitus there are a variety of coetaneous manifestations. Good metabolic control may prevent some of these manifestations and may support cure. Unfortunately, most glucose-lowering drugs also have coetaneous side effects.\(^{(15)}\)

2.1.1 Non infectious skin manifestation: Common Non infectious skin finding in diabetes included: acanthosisnegregcan (AN), skin tag, and vitiligo, and necrobiosislipodica, diabetic dermopathy. \(^{(16)}\)

Diabetes Mellitus (DM): is group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both.\(^{(14)}\)

2.1.2 Pathophysiology of Skin Tags: Both insulin and IGF-1 stimulate the synthesis of androgens in the ovaries and testis and both inhibit hepatic synthesis of sex hormone binding globulin (SHBG), allowing for
higher level of free biologically active androgens, which directly contribute to the pathophysiology of (coetaneous papilloma (skin tag).\textsuperscript{17} Skin Tags are characterized by hyper keratinization chronic hyperinsulinemia leads to chronic elevation of non-stratified FFAs, which causes increased production of epidermal growth factor and decrease in production of IGFBP-3 locally, allowing an increase in free IGF-1 that promotes the proliferation of keratinocytes, furthermore, decrease IGFBP-3 reduces the binding affinity of retinoic acid for its receptors, thus reducing the normal inhibition of cellular proliferation.\textsuperscript{17}

2.2 Diagnostic criteria of Diabetes Mellitus

The diagnostic criteria for diabetes mellitus were modified by the expert committee to allow for earlier detection of the disease. Diagnostic criteria are following\textsuperscript{18}:

- Random plasma glucose $\geq 200$ mg/dl (11.1 mmol/L) + symptoms of diabetes
- Fasting plasma glucose $\geq 126$ mg/dl (7.0 mmol/L)
- Two-hour plasma glucose $\geq 200$ mg/dl (11.1 mmol/L) during an OGTT (75-g glucose load)

An intermediate group who did not meet the criteria of diabetes mellitus but who had glucose level above normal was defined by two methods:\textsuperscript{18}

- Fasting glucose levels $\geq 110$ mg/dl but $< 126$ mg/dl were called the impaired fasting glucose group
- Patients who had 2-hour OGTT levels of $\geq 140$ mg/dl but $< 200$ mg/dl was defined as impaired glucose tolerance
- Hemoglobin A\textsubscript{1C} is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a 2- to 3-month period of time. The test plays a critical role in the management of the patient with diabetes.\textsuperscript{19}
2.2.1 HbA1c testing in diagnosing diabetes

The World Health Organization (WHO) suggests the following diagnostic guidelines for diabetes:

HbA1c below 42 mmol/mol (6.0%): Non-diabetic

HbA1c between 42 and 47 mmol/mol (6.0–6.4%): Impaired glucose regulation (IGR) or Prediabetes.

HbA1c of 48 mmol/mol (6.5%) or over: Type 2 diabetes

2.3 Dyslipidemias

Dyslipidemias are disorders of lipoprotein metabolism, including lipoprotein overproduction and deficiency. These disorders may be manifested by elevation of the serum total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride concentrations, and a decrease in the high-density lipoprotein (HDL) cholesterol concentration. Abnormal blood lipids are an increasing health problem in the world. Studies from India have shown upward trend in the prevalence of dyslipidemia, even among the young adult population.

Dyslipidemia plays a crucial role in the development of cardiovascular diseases, which has become the leading cause of death in most developed as well as in developing countries. It is now known that dermatological disorders, such as psoriasis are associated with dyslipidemias. (20)

2.3.1 Dyslipidemia and skin: Many dermatological disorders are known to be associated with dyslipidemia. Most of these are chronic inflammation diseases, and underlying mechanism may involve secretion of pro inflammatory cytokines. Studies have shown an increased occurrence of dyslipidemia in skin disorders like psoriasis, lichen planus,
pemphigus, granuloma annulare, histiocytosis, and connective tissues diseases like lupus erythematosus\(^{(20)}\)

2.3.2 Lipid Profile

This group of tests measures the amount of cholesterol and other fats in your blood\(^{(18)}\).

- Total cholesterol
- Triglycerides, another type of fat that causes hardening of the arteries.
- Test results may vary depending on age, gender, health history, the method used for the test, and other things. Test results may not mean have a problem.

Results are given in milligrams per deciliter (mg/dl). Here are the ranges for total cholesterol in adults: \(^{(18)}\)

- Normal: Less than 200 mg/dl
- Borderline high: 200 to 239 mg/dl
- High: At or above 240 mg/dl

The above numbers are general guidelines, because actual goals depend on the number of risk factors you have for heart disease.

- High levels of triglycerides are linked with a higher heart disease risk.

Here are the adult ranges: \(^{(18)}\)

- Normal: Less than 150 mg/dl
- Borderline high: 150 to 199 mg/dl
- High: 200 to 499 mg/dl
- Very high: Above 500 mg/dl
2.4 Previous studies

Many current researches and studies in different countries and nations assessing the association between the skin tags and (Diabetes Mellitus and dyslipidemias)

In Europe 1987 there is study was conducted BY Kahana M, Grossman E, Feinstein A, Ronnen M, Cohen M, Millet MS in which the skin tag serve as marker for DM .which Two hundred and sixteen non hospitalized patients with skin tags (ST) were studied for the presence of diabetes mellitus (DM) and obesity. Overt DM was found in 57 (26.3%) patients and impaired glucose tolerance test was found in 17 (7.9%) patients. Sixteen new cases of DM (6%) were found among this group. All the diabetic patients in the study population had non-insulin dependent DM. Sixty-two (28.7%) of the patients were obese. No correlation was found between the localization, size, color and number of the ST and the presence of DM. this study indicates that ST are not associated with increased incidence of obesity compared to the general population. On the other hand, ST are associated with impaired carbohydrate metabolism, and may serve as means for identifying patients at increasing risk of having DM.(21)

In an Epidemiological study in India by DM Thappa :22-oct-1995 where 35 patients with ST were screened out of 5000 consecutive patients visiting dermatology clinic to ascertain whether skin tags (ST) are associated with a higher risk for diabetes mellitus (DM). The study group ranged in age from 35 to 73 years, of the cases, 62.8% (22 patients) had DM. Four new cases of DM (11.4%) were found among this group. All the diabetic patients in this study population had noninsulin dependent DM, The frequency of DM in ST patients was found to increase with age; however, it was statistically insignificant. No correlation was found
between localization, size, color, or number of ST and the presence of DM. This study confirmed that the frequency with which ST had been found to co-exist with DM in this population is significant, and ST may serve as a marker for DM.\(^{(22)}\)

In turkey at June 2002 there was other study by S. Demir.Y, evaluated 120 patients with acrochordon for the presence of impaired carbohydrate metabolism. Overt diabetes mellitus (DM) was found in (73.3\%) 88 patients, glucose intolerance was detected in(5\%) 6 patients and (3.3\%) 4 patients had reactive hypoglycemia. concluded that acrochordons may be skin markers of underlying impaired carbohydrate metabolism and the patients with acrochordon should be evaluated for the presence of diabetes mellitus.\(^{(23)}\)

In Tehran, Iran at nov.2007 A case-control study was conducted by AbbasRasi MD, RaziehSoltani-Arabshahi MD, NasimShahbazi MD in individuals over 15 years old, comparing cases (n = 104) with at least three skin tags and age-, sex-, and body mass index (BMI)-matched controls (n = 94) without skin tag. Cases and controls were recruited from patients consecutively seen at an academic outpatient dermatology clinic. All patients underwent a standard 2-h oral glucose tolerance test with 75 g glucose. The result of this study was that Patients with skin tag had higher frequency of diabetes than the control group (23.07\% vs. 8.51 \%). The difference in the frequency of IGT was not significant (13.46\% vs. 10.63\%), there was a positive correlation between the total number of skin tags and the mean fasting plasma glucose, and patients with more than 30 skin tags were particularly at an increased risk of diabetes (52.0\%). No correlation was found between the number of skin tags and BMI. We did not find any correlation between the anatomical localization of skin tags and impaired carbohydrate metabolism, except for skin tags
under the breast in women. These results show an increased risk of diabetes mellitus in patients with multiple skin tags. With regard to the importance of early diagnosis of diabetes, we recommend a high level of suspicion for impaired carbohydrate metabolism in patients with skin tag.\(^{(24)}\)

In Germany 2008 there was other study by Sudy E, Urbina F, Maliqueo M, Sir T involved the following, Clinical and metabolic glucose/insulin characteristics of men with multiple (8 or more) skin tags on the neck were compared with a control group with few or none. Both groups were divided in two subgroups according to normal or abnormal laboratory findings. In the study subgroup with normal laboratory findings the number of skin tags varied from 8-33, whereas in those with abnormal laboratory findings the range was 9-65. Eight or more skin tags were related with statistically significant laboratory glucose/insulin abnormalities: basal hyperinsulinemia (p<0.002), postprandial hyperinsulinemia (p<0.003), and postprandial hyperglycemia (p<0.01). In the multiple skin tag group 77 % had diverse laboratory abnormalities, including insulin resistance, basal hyperinsulinemia, postprandial hyperinsulinemia, glucose intolerance or type 2 diabetes, in contrast with the control group, where only 33 % showed laboratory abnormalities. One-third of the study group had acanthosisnigricans. Only 15 % of patients with metabolic abnormalities did not show any cutaneous expression of glucose/insulin alterations (9 or more skin tags on the neck, acanthosisnigricans, or waist circumference greater than 95 cm). Multiple skin tags were more sensitive than acanthosisnigricans in identifying those with alterations in the glucose/insulin metabolism (77 vs. 32 % respectively), although less specific (68 vs.100%). Multiple skin tags should raise suspicion of insulin resistance or hyperinsulinemia.\(^{(25)}\)
In Brazil 2010 a cross-sectional study involving adult patients receiving care at a university teaching hospital was conducted by Tamega Ade A, Aranha AM, Guiotoku MM, Miot LD, Miot HA to evaluate the association between skin tags in the neck or axillary regions and insulin resistance. Cases were defined as patients with > 5 skin tags in the neck region and/or axilla. Insulin resistance was estimated using the HOMA-IR index. Results were adjusted for the other known covariates of risk for insulin resistance using a multiple logistic regression model. Ninety-eight cases and 103 controls were evaluated. There was no difference between the groups with respect to age or gender. Skin tags were directly associated with HOMA-IR values (odds ratio = 1.4), hypertriglyceridemia and body mass index, irrespective of adjustment for diabetes mellitus, age, skin phototype, gender, family history of diabetes mellitus or hip/waist ratio. Qualitatively elevated HOMA-IR levels (>3.8) were also significantly associated (odds ratio = 7.5). The presence of multiple skin tags was strongly associated with insulin resistance irrespective of other risk factors.\(^{(26)}\)

In Iran 2012 other study by Ramin Taheri, BatoolOodi, RahebGhorbani in the same task in whether there is association between skin tag and diabetes mellitus. This study was carried out on 80 patients with skin tags as a case group and 80 patients without skin tags as a control group that they were referred to Semnan dermatological clinics. Then fasting blood sugar (FBS) were checked out in both two groups. In addition, height and weight were measured in all patients and body mass index (BMI) calculated for each of the patient. Results: 43.8% and 55% of patients were respectively female in the case group and the control group. Age mean (± SD) was 44.3±16.6 and 37.3±18.9 years in the case and control group, respectively. BMI mean (±SD) index was 28.0±4.3kg/m² in the patients with skin tag, whereas, it was 25.5±5.1 kg/m² in the patients
without skin tag (P=0.001). Patients with skin tag had higher frequency of diabetes than patients in the control group (27.5% vs. 5%) and also the case group showed a higher frequency of pre diabetes than the control group (20% vs. 15%). The probability of presence of diabetes mellitus in the patients with skin tag was 6.82 times more than the patients in the control group (Odds ratio=6.82, 95% Confidence interval: 2.06-22.56, P=0.002). These data suggest that there was an association between skin tag and diabetes mellitus. Therefore, screening of patients with skin tag is recommended for early diagnosis diabetes mellitus.\(^{(27)}\)

This study done by Guy’s, St Thomas’s, report details four patients who had skin tags, mainly on their torso, neck, and axillae, and who also displayed an abnormal lipid profile. All showed an increased serum triglyceride (fasting > 1.70 mmol/litre) and decreased highdensity lipoprotein (HDL) cholesterol (< 1.1 mmol/litre in women and 1.0 mmol/litre for men) concentration. The displayed lipid profile is also known as the atherogenic profile and is associated with insulin resistance, type 2 diabetes mellitus, and an increased risk of cardiovascular disease. Two of the patients had impaired glucose tolerance and one had type 2 diabetes mellitus. Three of the individuals had coronary artery disease. Skin tags might be a useful clinical sign that could alert clinicians to screen such individuals for abnormal lipids, type 2 diabetes mellitus, and cardiovascular disease.\(^{(28)}\)

A comparison study of lipid profile levels between skin tags affected people and normal population in Tehran done by Abbas Rasi,\(^1\) Alireza Faghihi,\(^2\) Yaser Rahmanzadeh,\(^3\) and Habib Hassannejad, Iran from April 2009 to June 2011, 168 patients enrolled the study: Sixteen patients were lost to follow-up for reasons unrelated to the study. Among the remaining 152 patients, there were 89 females (58.5%) and 63 males (%41.5). The
age ranged between 18 and 73 years (mean age, 49.6 years). Based on the TLGS study, 136 men and 220 women enrolled the control group of the study. The mean age was 28.4 years. No clinically significant differences were found in demographic variables between cases and control group. Mean skin tag number was 12.6 per subject. In 56 patients (36.8%), skin tag number was low (<10). In 75 subjects (51.9%) it was moderate (between 10 to 30) and finally in 17 patients (11.1%), total body skin tags number was high (≥30). Statistical analysis showed no significant differences between skin tag number and hypertriglyceridemia or hypercholesterolemia. (29)
Chapter Three
3. Materials and Methods

3.1 Study design:
This is comparative case control study.

3.2 Study area:
The study was conducted at Khartoum state (Alanood hospital).

3.3 Study duration:
The study was conducted during the period from March to July 2018.

3.4 Study population and sample size:
All population with skin tags (at least three skin tags) in different age and gender selected randomly (50 patients with skin tags (28 male and 22 female)) and (20 healthy individual (11 male and 9 female)).

3.5 Inclusion criteria:
Patient with at least three benign diagnosed skin tags.

3.6 Exclusion criteria:
Pregnant women, acromegaly, poly cystic ovary syndrome, insulinoma, drugs that induced hyperinsulinemia and hereditary skin tags were excluded.

3.7 Tool of data collection: Questionnaire: data were collected using well structured questionnaire make with patient face to face (age, gender, number of skin tags, location of skin tags, medical history).

3.9 Ethical clearance: All participants in the study had to sign consent for participation and the results of investigations were to be conveyed to their treating doctors who will discuss results with them and provide the necessary treatment.
3.10 **Samples collection:** Under a septic condition Fasting Venous blood (2.5 ml) were collected in fluoride oxalate container for fasting blood glucose, cholesterol and triglycerides and 2.5 ml venous blood in EDTA for HbA1c.
3.11 Methodology
3.11.1 Glucose method
Principle of the method; Glucose oxidase peroxidase

Glucose in the sample originate, by means of coupled reaction, acoloured complex that can be measured by spectrophotometry.

Procedure
1. Reagent were brought to room temperature.
2. Were pipetted into test tubes.
3. Mixed thoroughly and incubated the tube for 10 minutes at room temperature.
4. The absorbance of the standard and sample were measured at 500 nm against the blank.

3.11.2 HbA1c:
Principle
The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.
The more antigens in sample forms the more antigen-antibody complex and lead to stronger intensity of fluorescence signal on detector antibody.
Instrument for ichroma tests displays the content of glycated hemoglobin in terms of percent of the total hemoglobin in blood.

Procedure
1. .1 ml of hemolysis buffer were drawn and transferred it into detection buffer tube.
2. .005 ml of blood were drawn using capillary tube and was putting it into detection buffer tube.
3. The lid of the detection buffer tube were closed and was mixing the sample thoroughly by shaking it about 15 times.

4. .075 ml of the sample mixture was pipetted and loaded into sample well in the test cartridge.

5. Wait till sample mixture flow appears in the windows (about 10 seconds).

6. The cartridge was inserted into i-chamber slot.

7. The cartridge was leaved into i-chamber for 12 minutes before removing. Scan the sample-loaded cartridge immediately when the incubation time is over.

8. The test result was red on the display screen of the instrument for ichroma test.

3.11.3 Cholesterol method;
Cholesterol oxidase\peroxidase (Bio Systems)

**Principle**
Free and esterified cholesterol in the sample originates by means of the coupled reactions, a coloured complex that can be measured by spectrophotometry.

**Procedure**
1. Reagent were brought to room temperature.
2. The reagent were pipetted in to labelled test tubes.
3. The tube was mixed thoroughly and incubated for 10 minutes.
4. The absorbance of the standard and sample were measured at 500nm against a blank.

5.

3.11.4 Triglycerides method
Glycerol phosphate oxidase\peroxidase (Bio Systems)

**Principle of the method**
Triglycerides in the sample originates, by means of the coupled reactions, coloured complex that can be measured by spectrophotometry.
Procedure
1. The reagent were brought to room temperature.
2. Reagent were pipetted into labelled test tube.
3. The tube was mixed thoroughly and incubated for 10 minutes.
4. The absorbance of the standard and sample were measured at 500 nm against blank.

3.12 Data Analysis:
Data were analyzed using computerized program SPSS (T test).
Chapter Four
4. Results

Table (4.1) Comparison between the mean of fasting glucose, HbA1c, Cholesterol and Triglycerides in patients with skin tags and in healthy individuals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Study groups</th>
<th>No</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Mg dl</td>
<td>Case</td>
<td>50</td>
<td>111</td>
<td>16.1</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>86</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>HbA1c %</td>
<td>Case</td>
<td>50</td>
<td>5.8</td>
<td>0.6</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>20</td>
<td>5.0</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Cholesterol Mg dl</td>
<td>Case</td>
<td>50</td>
<td>205</td>
<td>27.8</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>20</td>
<td>149</td>
<td>48.7</td>
<td></td>
</tr>
<tr>
<td>Triglycerides Mg dl</td>
<td>Case</td>
<td>50</td>
<td>160</td>
<td>34.0</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>20</td>
<td>170</td>
<td>34.3</td>
<td></td>
</tr>
</tbody>
</table>
Table (4.2) Comparison between the mean of fasting glucose, HbA1c, Cholesterol and Triglycerides with different number of skin tags.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No of skin tag</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Mg dl</td>
<td>1-10</td>
<td>108</td>
<td>14.0</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
<td>121</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>HbA1c %</td>
<td>1-10</td>
<td>5.6</td>
<td>0.4</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
<td>6.2</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Cholesterol Mg dl</td>
<td>1-10</td>
<td>200</td>
<td>29.3</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
<td>219</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>Triglycerides Mg dl</td>
<td>1-10</td>
<td>161</td>
<td>35.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
<td>159</td>
<td>31.9</td>
<td></td>
</tr>
</tbody>
</table>
Table (4.3) comparison between the frequency and percentage of gender and number of skin tag.

<table>
<thead>
<tr>
<th>Gender</th>
<th>No of skin tag</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-10</td>
<td>11-20</td>
</tr>
<tr>
<td>Male</td>
<td>Frequency</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>62.2%</td>
</tr>
<tr>
<td>Female</td>
<td>Frequency</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>37.8%</td>
</tr>
</tbody>
</table>
Table (4.4) comparison between the frequency and percentage of age and number of skin.

<table>
<thead>
<tr>
<th>Age</th>
<th>No of skin tag</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-10</td>
<td>11-20</td>
</tr>
<tr>
<td>20–40 years</td>
<td>Frequency</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>48.6%</td>
</tr>
<tr>
<td>41–80 years</td>
<td>Frequency</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>51.4%</td>
</tr>
</tbody>
</table>
Chapter Five
5.1 Discussion

A total of 50 cases: [28 (56%) male and 22(54%) female] and a total of 20 healthy control [11(55%) male and 9(45%) female]. All the results were expressed as mean+ or – SD value. The fasting blood glucose was higher in patients with skin tags and was statistically significant [p value <0.05] shows in table 1, The mean of case (111mg/dl) is higher than mean of control (89mg/dl). The overall patients [34(69%)] were impaired FBG according to WHO criteria.

The HbA1c in case was higher in patients with skin tags and was statistically significant [p value <0.05] shows in table 1, the mean of case (5.8%) is higher than mean of control (5%).

These results of FBG and HbA1c go in accordance with the finding of other study which has found a relationship between Skin Tags and diabetes mellitus.\(^{(22, 23, 24, \text{ and } 25)}\)

The total cholesterol in case is higher in patients and was statistically significant [p value <0.05], the triglycerides in case is less than control and was not statistically significant [p value >0.05] shows in table (4.1), these result go accordance of other study which found relationship between Skin Tags and dyslipidemia\(^{(28)}\) and discordance of study which found no relationship between Skin Tags and dyslipidemia.\(^{(29)}\)

There was statistically significant correlation between mean of FBG, HbA1c and total cholesterol with number of Skin Tags, and no significant correlation between triglycerides and number of Skin Tags, as shown in table (4.2). There was statistically significant correlation between gender and number of skin tags, as shown in table (4.3). There is no significant correlation between age and number of Skin Tags, as shown in table (4.4).

The most frequent localization of acrochordon was face and neck.
5.2 Conclusion

This study concludes that;

- There was statistically significant association between Skin Tags with glucose, HbA1c and cholesterol levels.
- There was statistically significant association between number of Skin Tags with glucose, HbA1c and cholesterol level.
- There was statistically significant correlation between gender and number of skin tags.
5.3 Recommendation

This study recommendes that:

- Patients with Skin Tags need suitable interventions like change in dietary habits.
- Patients with Skin Tags should have been screened for the presence of Diabetes Mellitus and Dyslipidemia.
- Others studies should be done with large sample size to approve this relationship.
Chapter Six
6.1 References

13. American Diabetes Association. living with diabetes, skin complication Copyright 1995-2013
20. Dr. Manjunath Mala Shenoy, Department of Dermatology, Yenepoya Medical College, Yenepoya University, Deralakatte, Mangalore - 575 018, Karnataka, India. E-mail: ni.oc.oohay@711675htanujnam


28. Department of Chemical Pathology, Guy’s, St Thomas’s, University Lewisham Hospital, London SE13 6LH, UK

29. Abbas Rasi,1 Alireza Faghihi,2 Yaser Rahmanzadeh,1 and Habib Hassannejad
6.2 Appendixes

Appendix I

بسم الله الرحمن الرحيم

Ministry of Higher Education and Scientific Research
University of Shendi
Faculty of Graduate studies and Scientific Research
Assessment of Fasting Blood Glucose, HbA1c and Lipid profile in Patients with Skin Tags at Khartoum state

Questionnaire

Patient ……………………………………… Lab No ……………
Age………………
Gender Male ( ) female ( )
Numbers of Skin Tags …………………………………
Locations of Skin Tags ………………………
Previously diagnosed with …. Diabetes ( ) prediabetes ( ) none ( )
Type ………
Pregnant ( )
Acromegaly ( ) hereditary skin tags ( ) insulinoma ( )
Drugs;
Telephone NO ……………….Signature ……………...

Parameters
FBS = ……. mg/dl
HbA1c = ……. %
Cholesterol= ……. mg/dl
Triglycerides = ……. mg/dl
Appendixes II:
Acrochordons (skin tags)
PRINCIPLE OF THE METHOD

Triglycerides in the sample, together with the reagents of the coupled reactions described below, are determined spectrophotometrically. The following coupled reactions are used:

1. Triglycerides + H₂O → Glycerol + Fatty acids
2. Glycerol + ATP → Glycerol-3-P + ADP
3. Glycerol-3-P + 2P → Glycerol-3-P-P → Diacylglycerol
4. Diacylglycerol + H₂O → Diglyceride + Fatty acid

CONTENTS

<table>
<thead>
<tr>
<th>COD 11269</th>
<th>COD 11260</th>
<th>COD 11268</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 30 mL</td>
<td>1 x 50 mL</td>
<td>1 x 200 mL</td>
</tr>
</tbody>
</table>

COMPOSITION

4. Reagent Pipette 45 mmol, magnesium chloride 5 mmol, 4-chlorophenol 8 mmol, pH: 7.0
5. Triglycerides Standard: Glycerol equivalent to 200 mg/mL, pH 7.0

STORAGE

Store at 2-8°C

REAGENT PREPARATION

- Reagents are supplied ready to use.

ADDITIONAL EQUIPMENT

- Thermometer: Water bath at 37°C
- Spectrophotometer or photometer able to read at 502 ± 20 nm

SAMPLES

Serum or plasma collected by standard procedures.

PROCEDURE

1. Bring the Reagent to room temperature
2. Pipette into labeled test tube (Note 1)
3. Mix thoroughly and incubate the tubes for 15 minutes at room temperature (10-30°C) or 5 minutes at 37°C
4. Measure the absorbance (A) of the Standard and Sample at 502 nm against the Blank.

CALCULATIONS

The triglyceride concentration in the sample is calculated using the following general formula:

\[ \text{Triglycerides (mg/dL)} = \left( \frac{A_{\text{sample}} - A_{\text{blank}}} {A_{\text{standard}} - A_{\text{blank}}} \right) \times (\text{Concentration of Standard}) \]

If the Triglycerides Standard provided has been used to calibrate (Note 2):

\[ A_{\text{blank}} = x \times \text{mg/dL triglycerides} \]

REFERENCE VALUES

The following values of serum triglycerides have been established by the US National Institutes of Health and have also been agreed upon by other authorities for use in the evaluation of risk:

- Normal: <100 mg/dL
- Borderline-high: 100-199 mg/dL
- High: ≥ 200 mg/dL

QUALITY CONTROL

It is recommended to use the Biochimica Clinical Serum Control (lots 10101, 10102, 10103, 10104) and 10105, 10106, 10107, 10108, 10109, 10110 to verify the performance of the reagents and procedures.

METROLOGICAL CHARACTERISTICS

- Detection limit: 1.8 mg/dL
- Linearity limit: 600 mg/dL
- For higher values dilute sample 1:4 with distilled water and repeat measurement

- Reproducibility (within run):
  - Mean concentration: CV = 1.2% ± 0.5%
  - Variability: CV = 2.6% ± 1.1%

- Reproducibility (between runs):
  - Mean concentration: CV = 1.7% ± 0.5%
  - Variability: CV = 1.3% ± 0.5%

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2).

Notes:
1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibrations with the provided standards may cause a matrix-related bias, especially in some analysers. In these cases, it is recommended to calibrate using a serum based standard (Biochimica Clinical, lot 10111).

BIBLIOGRAPHY

5. Fireman and Foxman, Effects of illness on clinical laboratory tests, 4th ed. AACC Press, 2001
PRINCIPLE OF THE METHOD

Free and esterified cholesterol in the sample undergoes, by means of the coupled reactions described below, a saturated compound that can be measured by spectrophotometry.

CHOLESTEROL

Contents

A. Reagent 1
B. Standard

Composition

A. Reagent 1
C. 33 mmol/L sodium hydroxide
D. 5.5 mmol/L phenol
E. Cholesterol - 50 mg/L

Storage

Store at 2-8°C.

Reagents and standards are stable until the expiry date shown on the label when stored tightly closed and protected from light.

Reagents

A. Reagent 1: Sodium hydroxide (33 mmol/L), phenol (5.5 mmol/L), cholesterol solution (50 mg/L) in water.

Sensitivity

200 mg/dl ± 10 mg/dl

Reagent Preparation

Reagents and standards are provided ready to use.

Additional Equipment

- Spectrophotometer or photometer capable of reading at 550 ± 20 nm.

Samples

Serum or plasma collected by standard procedures.

Procedure

1. Add the Reagent 1 to the sample.
2. Add the Standard (1:10).

Calculations

Cholesterol concentration in the sample is calculated using the following relationship:

\[ C = \frac{A_{	ext{sample}} - A_{	ext{blank}}}{A_{	ext{standard}} - A_{	ext{blank}} \times \text{Concentration}} \]

REFERENCE VALUES

The following values are limits that have been established by the US National Cholesterol Education Program and have been designed to reduce the incidence of coronary artery disease.

- Low: 125 mg/dl
- High: 200 mg/dl
- Very high: 265 mg/dl

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum (Brand I, II, III, and IV) to verify the performance of the measurement procedure.

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.2 mg/dl
- Linearity limit: 1900 mg/dl ± 20 mg/dl.
- For higher values dilute sample 1:2 with distilled water and re-measure.
- Repeatability (RSD %)
  - Mean Concentration: 0.5 %
  - 200 mg/dl ± 5.5 mg/dl:
  - 0.5 %
  - 20 mg/dl ± 10 mg/dl:
  - 1 %

- Reproducibility (RSD %)
  - Mean Concentration: 0.5 %
  - 200 mg/dl ± 5.5 mg/dl:
  - 0.5 %
  - 20 mg/dl ± 10 mg/dl:
  - 1 %

DIAGNOSTIC CHARACTERISTICS

Cholesterol is a form of high molecular weight and possesses the oxygendependent cholesterol esterification.

Cholesterol is a major source of energy and is also synthesized by the liver and other tissues. Cholesterol is transported in plasma by lipoprotein.

BIBLIOGRAPHY

GLUCOSE

GLUCOSE OXIDASE/PEROXIDASE

PRINCIPLE OF THE METHOD
Glucone from the sample organisms, by means of the coupled enzymatic reactions described below, a series of compounds that can be measured by spectrophotometry.

Glucose + OH₂ → Glucuronic acid

Glucose dehydrogenase

2-14H₂O 4-14H₂O + H₂O

Glucuronic acid

CONTENTS
C00 11095
C00 11096
C00 11098
C00 11099
C00 11100

A. Reagent
B. Standard
C. Buffer
D. Enzyme
E. Chromogen

COMPOSITION
A. Reagent: Peroxidase 50 mg/mL, phenol 0.5% w/v, glucose oxidase > 100 U/mL, paranitrophenyl 1 U/mL, potassium hydroxide 0.5 mol/L, pH 7.5.
B. Glucose/Dehydrogenase Standard: Glucose 100 mg/mL, (0.55 mg/mL), wash 50 mg/mL, containing 5% DMSO, aqueous primary standard.

STORAGE
Store at 2-8°C

REAGENT PREPARATION
Reagent and Standard are provided ready-to-use.

ADDITIONAL EQUIPMENT
- Thermocentric water bath at 37°C
- Hach® spectrophotometer or photometer table model at 540 ± 20 nm

SAMPLES
Samples are obtained according to standard procedures. Blood sample must be obtained from the tail tip or ear. The sample is boiled in a boiling water bath until the glucose is completely precipitated. Glucose is measured in plasma or serum by a method based on the colorimetric reaction of glucose with 2,4-DNP.

PROCEDURE
1. Wash the reagents in room temperature
2. Pipette into labeled test tubes (Note 1):
   - Glucose Standard (50 mg/mL)
   - Sample

3. Mix thoroughly and incubate the tubes for 15 minutes at room temperature (15-25°C) or for 5 minutes, at 37°C
4. Measure the absorbance (A) of the Standard and the Sample at 500 nm against the Blank (Note 2)

CALCULATIONS
The glucose concentration in the sample is calculated using the following general formula:

A - A blank

A Standard

REFERENCE VALUES
- Normal, fasting: 0.05-0.09 mg/mL
- Diabetic, normal: 0.05-0.09 mg/mL
- Diabetic, fasting: 0.05-0.09 mg/mL

QUALITY CONTROL
- Standards: Levels 0.2 mg/mL, 0.5 mg/mL
- Unacceptably: 0.3 mg/mL, 0.8 mg/mL. For higher values, dilute sample 1:4 with distilled water and repeat measurement.

BIBLIOGRAPHY