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A PROSPECTIVE OPEN CONTROLLED STUDY ON NON-SEGMENTAL VITILIGO PATIENTS FOCUSING ON THE METABOLIC CHANGES PARTICULARLY CHOLESTEROL IN BLOOD AND SKIN TISSUES

By

Ahmed W. Ismail*, Shaker M. Ezz Eddin*, Ahmed H. Nouh*, and Mohamed S. Al-Shorbaji**

*Departments of Dermatology and Clinical Pathology, AL-Azhar Faculty of Medicine

Corresponding Author: Ahmed Waheed Ismail,

Mobile: +201017903781, **E-mail:** dr.waheed76@gmail.com

ABSTRACT

Background: Vitiligo is an acquired idiopathic skin disease that appears as skin white depigmented patches due to loss of melanocyte cells that are responsible for melanin pigment production which gives color to the skin, hairs, retina and mucous membranes.

Objective: To assess the metabolic changes (namely cholesterol) in blood and tissues of patients suffering from non-segmental vitiligo.

Patients and Methods: Our study was carried out during the period from September 2020 to March 2021. Patients were selected from the outpatient skin clinic at Al-Hussein Hospital, Al-Azhar University, Cairo. A total number of 30 patients suffering from non-segmental vitiligo were included in addition to 30 normal persons of the same age group and both sexes, not having any skin disease, not suffering from any systemic morbid disease (diabetes mellitus, dyslipidemia, liver disease, kidney disease or hypertension) or taking any systemic medication for any other disease were introduced in the study as a control group.

A Punch skin biopsy was taken from the vitiliginous patches and another one from the adjacent apparently normal skin of the patients. The specimens were stained with special stains for cholesterol (Sudan III) stain.

Results: The results of this study disclosed elevated levels of cholesterol in the blood samples of about 21 patients (70%) among the total number of patients, compared to 9 patients (30%) who showed normal blood cholesterol values.

Tissue biopsy specimens from the vitiliginous patches of the patients, stained with Sudan III stain, disclosed the presence of cholesterol in the dermis in 22 patients (73.3 %), while the rest of the specimens of 8 patients (26.7 %) showed no cholesterol deposition.

Tissue biopsy specimens from the apparently normal skin of the patients did not show any lipids in the dermis of these samples.

Conclusion: Increased blood cholesterol levels in addition to the presence of cholesterol (as a secondary metabolic product) deposited in the skin tissue samples of vitiliginous patches could be related to nerve fibers which were undergoing a degenerative process. These nerve fibers were innervating the melanocyte cells in the skin.

Keywords: Cholesterol, Tissue, Vitiligo.

INTRODUCTION

Vitiligo is an acquired, progressive disorder of pigmentation characterized by the development of well-defined depigmented macules on the skin *(Ezzedine et al., 2015)*. It is the most frequent cause of depigmentation with an estimated prevalence of 0.1 to 2 percent in both adults and children *(Krüger and Schallreuter, 2012)*.

Although, many theories have been debated for the etiology of vitiligo, the exact cause is still unknown. Genetic predisposition, autoimmunity, biochemical substances, viral infection, melanocyte self-destruction oxidative stress, neural degenerative theory are the most common hypotheses in the literature *(Alikhan et al., 2012)*.

The clinical course of vitiligo is variant and lesions may remain stable or progress slowly for years. Vitiligo Area Severity Index (VASI) and Vitiligo Disease Activity Score (VIDA) may be used for the assessment of the disease severity and activation *(Lakhani et al., 2016)*.

There is no one single permanent cure for the disease. Some treatment modalities complete may help progression of lesions and induce varying degrees of re pigmentation *(Iannella et al., 2016)*.

Blood cholesterol levels changes and tissue depositions as a sequlelae of neuronal degeneration correspond to existing evidence that cholesterol is necessary for normal neuronal function and morphology. Both a lack and an excess of cholesterol impair these features *(Orth and Bellosta, 2012)*.

The present work aimed to assess the metabolic changes (namely cholesterol) in blood and tissues in non-segmental vitiligo patients.

PATIENTS AND METHODS

All patients were subjected to complete personal, family and medical history:

Dermatological examination to evaluate was done the extend of lesions, sites and distribution and skin type.

- Serum cholesterol level will be assessed.
- Biopsy from affected skin patch and from non-affected skin around to evaluate presence of cholesterol using special stain.

Tissue staining by Supply Sudan III stain:

Fixation: Fresh tissue

Technique: Frozen tissue sections cut at 8 microns on adhesive slides

Staining procedure:

1. Frozen section slides were fixed in Formalin 10%, Phosphate Buffered for 1 minute.
2. Sections were rinsed carefully in two changes of distilled water.
3. Rinse was in Alcohol and Ethyl Denatured (70%).
4. Sections were stain in Sudan III, Saturated Alcoholic for 10 minutes, and tightly kept capped to avoid evaporation.
5. Differentiation was in alcohol, Ethyl Denatured (70%).
6. Sections were washed thoroughly in distilled water.

7. Sections were counterstained with Hematoxylin Stain and Mayer Modified for 2-3 minutes.
8. Sections were washed gently in several changes of tap water.
9. Sections were blue in Lithium Carbonate, Saturated Aqueous or Scott tap water Substitute for 10 dips. The use of a bluing agent was optional.
10. Sections were washed gently in several changes of tap water.
11. Excess water was blotted from slide; coverslip with Mount-Quick Aqueous Mounting Medium. Minimal pressure was used when applying coverslip or fat/lipid staining may be disturbed.

Statistical analysis: Data were analyzed using Statistical package for the Social Science (SPSS) version 24. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- For independent-samples: t-test was used.
- Fisher's exact test was used to compare qualitative data.
- P-value <0.05 was considered significant.
- Use Mann-whitny test for cholesterol.

RESULTS

There was a statistical significant difference between patients and control as regards serum cholesterol values **(Table 1)**.

Table (1): Comparison between patients and controls as regards serum cholesterol values

Parameters \ Groups		Patients (N = 30)		Control (N = 30)		P-value
Cholesterol level	**Mean ±SD**	224.1 ± 54.6		164.6 ± 224.6		**< 0.001**
	Range	130 - 330		124 - 198		
Cholesterol	**Normal**	9	30%	30	100%	**< 0.001**
		157. ± 23.01		164.6 ± 224.6		
	Hyper	21	70%	0	0%	
		252.9 ± 34.9		--------		

There was a statistically significant difference between vitiliginous tissue and non- vitiliginous tissue as regards cholesterol positivity **(Table 2)**.

Table (2): Comparison between vitiliginous biopsy skin tissue and non-vitiliginous biopsy skin tissue as regards cholesterol positivity

Cholesterol positivity \ Groups	Vitiliginous (N = 30)		Non-Vitiliginous (N = 30)		P-value
Negative	8	26.7%	30	100%	**< 0.001**
Positive	22	73.3%	0	0%	

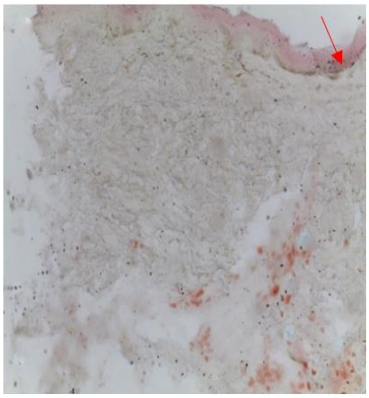

Figure (1): A 63 years old female with vitiliginous lesion biopsy taken from affected skin and stained with Sudan III shows positivity of cholesterol in dermis and arrow refer to red fat with blue nuclei

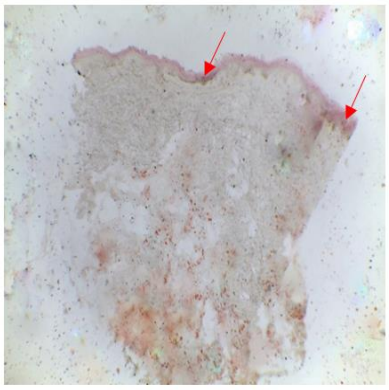

Figure (2): A 68 years old female with vitiliginous lesion biopsy taken from affected skin and stained with Sudan III shows positivity of cholesterol in dermis and arrows refer to red fat with blue nuclei

Activa

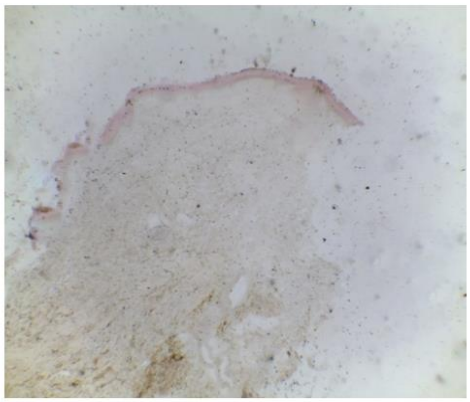

Figure (3): A 50 years old female with vitiliginous lesion biopsy taken from affected skin and stained with Sudan III shows negativity of cholesterol in dermis

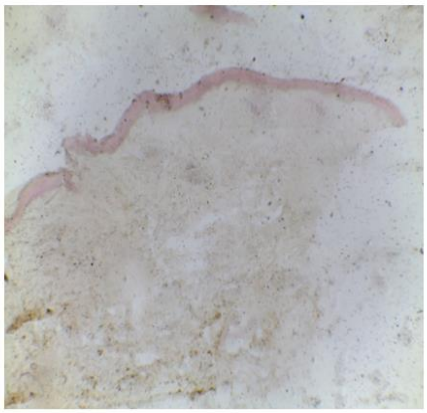

Figure (4): A 55 years old male with vitiliginous lesion biopsy taken from affected skin and stained with Sudan III shows negativity of cholesterol in dermis

DISCUSSION

Vitiligo is a disease known for thousands of years, the exact pathogenic mechanism is still unknown. Many mechanisms such as autoimmunity, genetic factors and involvement of inflammatory cytokines *(Kumar et al., 2014).*

Pillaiyar with co-authors in his work about signalling pathways inhibitors of melanogenesis mention based on previous research references, that many lipid-lowering agents were found to increase melanogenesis *(Pillaiyar et al., 2017).*

Cholesterol oxidation products have important physiological/ pathophysiological roles, including cholesterol homeostasis and oxysterols-induced cell death that can take part in degenerative pathologies *(Bellei et al., 2013).*

The study results of Raddatz with co-authors suggest that downregulation of cholesterol biosynthesis, by abnormally increased plasma and tissue cholesterol is a transcriptional marker for demyelination and quantitative loss of myelin-specific lipids *(Raddatz et al, 2016)*.

HDL cholesterol and apolipoproteins are accountable for low level degeneration, while LDL cholesterol and total cholesterol significantly worsen the neuronal degeneration *(Orth and Bellosta, 2012)*.

The level of total cholesterol in the blood was measured in all patients after 14 hrs of fasting. Hypercholesterolemia was observed in 21 patients (70%) while 9 patients (30%) revealed normal results of blood cholesterol.

Biopsy for comparison between vitiliginous and non-vitiliginous skin tissue was taken from all subjects and stained with Sudan III to detect cholesterol. Result showed highly statistical differences between two the types of samples. In vitiliginous tissue, 22 samples (73.3%) were positive for cholesterol depositions and 8 samples (26.7%) – negative, while in non-vitiliginous tissue all samples (100%) showed negative results.

CONCLUSION

Based on the data of this study, we can predict that increased blood and tissue cholesterol levels are sequelae of neuronal degeneration which in one of the proposed theories of Vitiligo pathogenesis.

The study (limitations were little sample size) of Patient; therefore, there is a need for more comprehensive studies with larger series and more participants to evaluate these links and their role.

Conflicts of interest: No conflicts of interest.

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تغيرات التمثيل الغذائى فى الدم والأنسجه فى مرضى البهاق غير المقطعى

محمد سعيد الشوربجى*، الدين احمد حسن نوح*، شاكر محمود عز*،

احمد وحيد اسماعيل

قسمى الأمراض الجلدية* و الباثولوجيا الاكلينيكيه **، كلية الطب، جامعة الأزهر

خلفيــة البحــث: البهــاق هــو مــرض مجهــول الســبب مكتســب يظهــر كبقــع بالجلــد ناقصــة الصــبغه، وهــذا الاضــطراب يحــدث بســبب فقــدان الخلايــا الصــباغية المســؤولة عــن إنتــاج صــبغة الميلانــين فــي الجلــد والشــعر والأغشــية المخاطيــه والشــبكية، حيــث تظهــر بقع بيضاء على أجزاء مختلفة من الجلد.

الهــدف مــن البحــث: دراســة وجــود الكوليســتيرول فــى الــدم وانســجة الجلــد بمرضــى البهاق ومقارنتها بالجلد الغير مصاب لنفس المريض.

المرضــي وطــرق البحــث: تــم إجــراء هــذه الدراســة علــى 30 شخصًــا يعــانون مــن مــرض البهــاق غيــر الثابــت عــلاوة علــى 30 فــردًا كمجموعــه تحكــم طبيعيــة لا يعــانون مــن البهــاق أو أي متلازمــة أيضــية مــن نفــس العمــر، وقــد تــم أخــذ عينــة مــن الجلــد المصــاب ومــن الجلــد غيــر المصــاب لتقيــيم وجــود الكوليســترول باســتخدام صــبغة خاصة (سودان 3).

نتــائج البحــث: أظهــرت دراســتنا وجــود زيــاده ال كوليســترول فــى الــدم فــي 21 مريضــا بنسبه 70%، بينما وجد 9مرضى بنسبه 30% بنسبة طبيعية.

وفيمــا يتعلــق بنتيجــة ترســب الكوليســترول فــي الأنســجة، هنــاك نتيجــة إيجابيــة فــي أنســجة البهــاق فــى 22 مريضــاً بنســبة 73.3%، بينمــا كانــت هنــاك نتيجــة ســلبية فــي أنســجة 8 مرضــى % 26.7 بينمــا فــي الأنســجة غيــر المصــابه بالبهــاق أظهــرت نتيجــة سلبية فى الكل العينات.

الاســتنتاج: زيــادة مســتويات الكولســترول فــي الــدم والأنســجة لهــا تــأثير ســلبي علــى تنكس الخلايا العصبية وهذه هي جزء من التسبب في البهاق.