<u>ORIGINAL RESEARCH</u>

A case-control clinical study for assessing the role of serum desnutrin as a biomarker of insulin resistance in subjects with vitiligo vulgaris

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ABSTRACT
Background: A common skin disorder affecting a large population is vitiligo which shows a characteristic feature of selective melanocyte destruction. Literature data is scarce concerning the reliable biomarkers of insulin resistance in vitiligo subjects. **Aim:** The present study aimed to assess the role of serum desnutrin as a biomarker of insulin resistance in subjects having vitiligo vulgaris. **Methods:** The study assessed 90 subjects with vitiligo vulgaris from both genders and tested against 90 controls that were gender and age-matched. In all the subjects, a comprehensive cutaneous and general examination was done along with the assessment of serum desnutrin, fasting serum insulin, LDL, VLDL, HDL, triglycerides, cholesterol, and FBG (fasting blood glucose). Also, HOMA-IR (Homeostasis Model Assessment + insulin resistance) was done for all the participants. **Results:** The study results depicted a statistically significant difference for serum desnutrin, HOMA-IR, fasting insulin, FBG, and HDL in subjects with vitiligo vulgaris compared to the controls with $p<0.001$. Also, a negative correlation was seen in desnutrin and HOMA-IR, fasting insulin, VLDL, LDL, and FBS with $p<0.05$. A positive non-significant correlation was seen in HDL and desnutrin levels with $p=0.06$. **Conclusion:** The present study concludes that suppressed serum desnutrin levels are seen in subjects with vitiligo vulgaris secondary to increased serum insulin and glucose levels leading to insulin resistance and hyperlipidemia depicting desnutrin as a biomarker of insulin resistance in these subjects.
Keywords: Desnutrin, insulin resistance, vitiligo, vitiligo vulgaris

INTRODUCTION

Vitiligo is a common skin disorder that is characterized by depigmentation along with selective melanocyte destruction. Vitiligo vulgaris affects the population globally with increasing evidence in the Indian population. The etiopathogenesis of vitiligo vulgaris has been attributed to various factors with a major link as an autoimmune disorder correlated with environmental and genetic predisposing factors combined with cell-detachment, oxidative stress, and metabolic anomalies making the etiopathogenesis of the disease.[1]

Vitiligo vulgaris affects nearly 0.5% to 2% of the global population. Previous literature data strongly suggest that vitiligo has various systemic presentations along with its effect on the skin. Vitiligo vulgaris has also been linked to various metabolic anomalies including lipid abnormalities and glucose intolerance. All of these factors together can be attributed to the systemic presentations in subjects with vitiligo vulgaris. It has been noted that metabolic syndrome can be prevented by the melanocytes seen in the adipose tissue as they can help reduce oxidative stress, oxidative damage, and inflammation.[2]

Desnurin is a protein family member that can help in the lipolysis in adipose tissues. Desnutrin is reversibly activated in the fasting state and is reduced after eating. Desnutrin is also known as ATGL (adipose triglyceride lipase). Desnutrin is primarily seen in the adipose tissues of the body and depicts high substrate, especially in the cases of TAG (triacylglycerol).[3] Desnutrin is considered a main lipase that catalyzes the hydrolysis of triacylglycerol in the adipose tissues of the body. The accumulation of triacylglycerol secondary to the ablation of desnutrin leads to the activation of adipocyte hypertrophy. Desnutrin overexpression leads to a reduction in the content of adipocyte TAG which further reduces the obesity induced by the diet.[4] Also, lipolysis, mediated by desnutrin, simulates the oxidation of fatty acid and their re-esterification within the adipocytes. Irrespective of the mechanism of alterations in adipose tissue lipolysis, free fatty acids are increased and TAG is stored. This can lead to various metabolic disorders including cardiovascular disorders, hypertension, type 2 diabetes mellitus, and insulin resistance.[5]

Subjects with vitiligo vulgaris are at higher risk of experiencing increased levels of insulin and glucose that could further decrease the serum desnutrin and can be attributed to being a cause of insulin resistance. Hence, the present clinical study aimed to assess the levels of serum desnutrin and its association with insulin resistance in subjects with vitiligo vulgaris.

MATERIALS AND METHODS
The present case-control clinical study was aimed to assess the levels of serum desnutrin and its association with insulin resistance in subjects with vitiligo vulgaris. The study assessed 90 subjects from both genders with vitiligo vulgaris that were tested against 90 gender and age-matched controls. Verbal and written informed consent were taken from all the subjects before study participation.

The inclusion criteria for the study were subjects that were of age 18 years or more, had vitiligo vulgaris, and were willing to participate in the study. The exclusion criteria for the study were subjects that did not give consent for study participation, lactating females, pregnant females, hypertensive, diabetics, obese, subjects under 18 years of age, and subjects with segmental vitiligo.

After the final inclusion of the study subjects, detailed history was recorded for all the subjects along with demographic data. This was followed by a clinical examination of all the subjects including a recording of their gender, age, occupation, education, habits, and marital status. In the disease history, vitiligo family history, previous vitiligo treatment, disease activity, and disease duration were assessed. Disease activity assesses the appearance of new lesions or the expansion of existing lesions. A general examination was done for all the subjects including BMI (kg/m2),

height in meters, and weight in kilograms (kg). In dermatological assessment, the activity of vitiligo, extension of vitiligo, anatomical site, and Fitzpatrick's phenotype were assessed. Vitiligo extension was assessed using the Vitiligo area scoring index[6] and vitiligo activity was assessed using the Vitiligo Disease activity score.[7]

After general, clinical, and dermatological examination, laboratory examination was done for the controls as well as study subjects. For laboratory testing, 5 ml of venous blood was collected from each subject under strict aseptic and sterile conditions. The collected blood was then allowed to clot for 30 minutes at room temperature followed by centrifugation at 2000-3000 rpm for 15 minutes. The supernatants were then collected and the serum was partitioned into three aliquots.

The first aliquot was used to assess VLDL (very low-density lipoprotein), LDL (low-density lipoprotein), HDL (high-density lipoprotein), TG (triglycerides), cholesterol, and FBG (fasting blood glucose). The second aliquot was used to assess the levels of fasting serum insulin. Insulin resistance was assessed using HOMA-IR (Homeostasis Model Assessment + insulin resistance) with fasting glucose level (mg/dl) X fasting insulin level (mU/ml)/405. Early insulin resistance and significant insulin resistance were assessed as HOMA-IR of ≤ 2.9 and > 2.9 respectively.[8] The third aliquot used for the detection of desnutrin was assessed using the quantitative ELISA technique with a desnutrin commercial human ELISA kit.

The data gathered were analyzed statistically using SPSS software version 21.0 (IBM Corp., Armonk, NY, USA) with Mann Whitney U test, Chi-square test, and Pearson correlation test. The data were expressed as mean and standard deviation. The level of significance was kept at $p < 0.05$.

RESULTS
The present cross-sectional, case-control, clinical study was aimed to assess the levels of serum desnutrin and its association with insulin resistance in subjects with vitiligo vulgaris. The study assessed 90 subjects from both genders with vitiligo vulgaris that were tested against 90 gender and age-matched controls. The mean age of the study participants was 37.85 ± 13.97 years in controls and 43.14 ± 14.74 years in cases which was non-significant with p=0.08. There were 51.1% (n=46) females and 48.8% (n=44) males in controls and 46.66% (n=42) females and 53.3% (n=48) males in cases which showed a non-significant difference with p=0.65. BMI of controls and cases was 25.97 ± 3.91 and 26.69 ± 3.31 kg/m2 (p=0.17). Height of controls and cases was 163.14 ± 7.19m and 164.12 ± 7.26 m respectively which was non-significant with p=0.37. The weight of study subjects and controls was 72.05 ± 11.35 and 69.31 ± 11.91 kg respectively showing a non-significant difference with p=0.15 (Table 1).

Table 1: Comparison of demographic data in two groups of study subjects

S. No	Characteristics	Controls		Cases		p-value
		n=90	%	n=90	%	
1.	Mean age (years)	37.85±13.97		43.14±14.74		0.08
2.	Gender					
3.	Females	46	51.11	42	46.66	0.65
4.	Males	44	48.88	48	53.3	
5.	BMI (kg/m2)	25.97±3.91		26.69±3.31		0.17
6.	Height (m)	163.14±7.19		164.12±7.26		0.37
7.	Weight (kg)	69.31±11.91		72.05±11.35		0.15

For clinical characteristics, the Koebner phenomenon was 0 in 88.8% (n=80) subjects and 1 in 11.1% (n=10) subjects. VIDA scores were -1, 0, 1, 2, 3, and 4 in 8.88% (n=8), 22.2% (n=20), 17.7% (n=16), 26.6% (n=24), 20% (n=18), and 4.44% (n=4) study subjects respectively. The mean VASI score in study subjects was 1.65±0.12. The mean vitiligo duration was 6.05±8.69 years. The cause was stationary and progressive in 15.5% (n=14) and 84.4% (n=76) study subjects respectively. The disease onset was gradual in 51.1% (n=46) subjects and was sudden in 48.8% (n=44) subjects respectively (Table 2).

Table 2: Disease-related data in study participants

S. No	Disease-related data	Number (n)	Percentage (%)
1.	Koebner phenomenon		
a)	0	80	88.8
b)	1	10	11.1
2.	VIDA		
a)	-1	8	8.88
b)	0	20	22.2
c)	1	16	17.7
d)	2	24	26.6
e)	3	18	20
f)	4	4	4.44
3.	Mean VASI	1.65±0.12	
4.	Mean duration	6.05±8.69	
5.	Cause		
a)	Stationary	14	15.5
b)	Progressive	76	84.4
6.	Onset		
a)	Gradual	46	51.1
b)	Sudden	44	48.8

On comparing various lipid variables in two study groups, it was seen that VLDL (very low-density lipoprotein), LDL (low-density lipoprotein), TG (triglycerides), and cholesterol levels were comparable in test and control subjects with respective p-values of 0.24, 0.08, 0.23, and 0.16. The HDL levels were significantly higher in subjects of the control group with 42.8±14.65 mmol/L compared to the test group where HDL levels were 38.89±4.22 mmol/L which was a statistically significant difference with p=0.01 as shown in Table 3.

Table 3: Comparison of lipid profile in control and test study subjects

S. No	Variables	Controls (n=45)	Tests (n=45)	p-value
1.	VLDL	19.64±3.6	21.89±6.83	0.24
2.	LDL	51.66±17.97	61.24±23.10	0.08
3.	HDL	42.8±14.65	38.89±4.22	**0.01**
4.	TG	97.04±19.37	145.27±163.14	0.23
5.	Cholesterol	124.6±88	120.26±24.42	0.16

On comparing Desnutrin, HOMA-IR, fasting insulin, and fasting blood glucose in two study groups, it was seen that desnutrin was significantly higher in control subjects with 19.56±4.7 ng/ml compared to 11.82±3.53 ng/ml in study group with p<0.001. HOMA-IR was significantly higher in test subjects compared to controls with p<0.001. Fasting insulin was significantly higher in the study group compared to controls with p<0.001. Fasting blood glucose levels were also higher in the test group compared to controls with p<0.001 as depicted in Table 4.

Table 4: Comparison of Desnutrin, HOMA-IR, fasting insulin, and fasting blood glucose in two study groups

S. No	Variables	Controls (n=45)	Tests (n=45)	p-value
1.	Desnutrin (ng/ml)	19.56±4.7	11.82±3.53	**<0.001**
2.	HOMA-IR	1.79±0.54	2.9±0.97	**<0.001**
3.	Fasting insulin (IU/ml)	9.29±2.45	12.33±3.59	**<0.001**
4.	Fasting blood glucose (mg/dl)	78.27±7.68	86.47±10.86	**<0.001**

The study results showed that for insulin resistance pattern significant resistance was seen in no subject from the control group and 32 subjects from the test group. This difference was statistically significant with p<0.001. Early insulin resistance was seen in 34 subjects from controls and 36 subjects from test groups which was significant with p<0.001. In total, there were 34 and 68 subjects respectively from control and test groups which was statistically non-significant with p=0.81 (Table 5).

Table 5: Comparison of insulin resistance pattern in two groups of study subjects

S. No	IR (insulin resistance)	Controls (n=45)	Tests (n=45)	p-value
1.	Significant resistance	0	32	**<0.001**
2.	Early resistance	34	36	**<0.001**
3.	Total	34	68	0.81

Concerning the correlation of study parameters and desnutrin levels in subjects with vitiligo, the correlation was statistically significant between desnutrin and HOMA-IR, fasting insulin levels, VLDL, LDL, and fasting blood glucose with respective p-values of <0.001, <0.001, 0.006, 0.01, and <0.001. However, the correlation of insulin resistance was non-significant with HDL, triglyceride, and cholesterol levels with p=0.05, 0.05, and 0.13 respectively as shown in Table 6.

Table 6: Correlation of study parameters and desnutrin levels in subjects with vitiligo

S. No	IR (insulin resistance)	Pearson correlation/ Spearman correlation	p-value
1.	HOMA-IR	-0.625	**<0.001**
2.	Fasting insulin	-0.583	**<0.001**
3.	VLDL	-0.256	**0.006**
4.	LDL	-0.213	**0.01**
5.	HDL	0.164	0.05
6.	TG	-0.173	0.05
7.	Cholesterol	-0.127	0.13
8.	FBG	-0.444	**<0.001**

DISCUSSION

It was seen that for clinical characteristics, the Koebner phenomenon was 0 in 88.8% (n=80) subjects and 1 in 11.1% (n=10) subjects. VIDA scores were -1, 0, 1, 2, 3, and 4 in 8.88% (n=8), 22.2% (n=20), 17.7% (n=16), 26.6% (n=24), 20% (n=18), and 4.44% (n=4) study subjects respectively. The mean VASI score in study subjects was 1.65±0.12. The mean vitiligo duration was 6.05±8.69 years. The cause was stationary and progressive in 15.5% (n=14) and 84.4% (n=76) study subjects respectively. The disease onset was gradual in 51.1% (n=46) subjects and was sudden in 48.8% (n=44) subjects respectively. These results were consistent with the studies of Kershaw EE et al[9] in 2006 and Langin D[10] in 2006 where authors reported similar clinical characteristics in subjects with vitiligo as in the present study.

The study results showed that on comparison of various lipid variables in two study groups, it was seen that VLDL (very low-density lipoprotein), LDL (low-density lipoprotein), TG (triglycerides), and cholesterol levels were comparable in test and control subjects with respective p-values of 0.24, 0.08, 0.23, and 0.16. The HDL levels were significantly higher in subjects of the control group with 42.8±14.65 mmol/L compared to the test group where HDL levels were 38.89±4.22 mmol/L which was a statistically significant difference with p=0.01. These findings were in agreement with the findings of Ergin C et al[11] in 2017 and Demir B et al[12] in 2014 where authors suggested comparable lipid profiles in subjects with vitiligo as seen in the present study.

On comparison of Desnutrin, HOMA-IR, fasting insulin, and fasting blood glucose in two study groups, it was seen that desnutrin was significantly higher in control subjects with 19.56±4.7 ng/ml compared to 11.82±3.53 ng/ml in study group with p<0.001. HOMA-IR was significantly higher in test subjects compared to controls with p<0.001. Fasting insulin was significantly higher in the study group compared to controls with p<0.001. Fasting blood glucose levels were also higher in the test group compared to controls with p<0.001. These results correlated with the results of Pietrzark A et al[13] in 2000 and Burge MR[14] in 2004 where authors suggested significantly higher desnutrin in controls compared to subjects with vitiligo.

The study results showed that for insulin resistance pattern significant resistance was seen in no subject from the control group and 32 subjects from the test group. This difference was statistically significant with p<0.001. Early insulin resistance was seen in 34 subjects from controls and 36 subjects from test groups which was significant with p<0.001. In total, there were 34 and 68 subjects respectively from control and test groups which was statistically non-significant with p=0.81. These findings were comparable to the studies of Birol A et al[15] in 2006 and Brunn JM et al[16] in 2003 where authors reported comparable insulin resistance in subjects with and without vitiligo which was similar to the present study.

On assessing the correlation of study parameters and desnutrin levels in subjects with vitiligo, the correlation was statistically significant between desnutrin and HOMA-IR, fasting insulin levels, VLDL, LDL, and fasting blood glucose with respective p-values of <0.001, <0.001, 0.006, 0.01, and <0.001. However, the correlation of insulin resistance was non-significant with HDL, triglyceride, and cholesterol levels with p=0.05, 0.05, and 0.13 respectively. These results were in line with the findings of Akrem J et al[17] in 2008 and Gopal KV et al[18] in 2007 where authors suggested desnutrin to have a significant role in vitiligo as in the present study.

CONCLUSION

Considering its limitations, the present study concludes that in subjects having vitiligo vulgaris owing to increased levels of serum insulin and glucose, decreased levels of serum desnutrin are seen which can be attributed to insulin resistance and hyperlipidemia. Hence, low levels of serum desnutrin can act as a biomarker of insulin resistance in subjects having vitiligo vulgaris. It is vital to have a multidisciplinary approach for early diagnosis of diabetes mellitus, hyperlipidemia, and insulin resistance in subjects having vitiligo vulgaris to prevent metabolic and cardiovascular complications.

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