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ORIGINAL ARTICLE

Role of Complement C3 as a predictor of NAFLD in Diabetic and Obese patients

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Abstract

Background: ***The complement system is increasingly acknowledged to be integrally tied to obesity and other numerous metabolic disorders related to it and may be involved in NAFLD.***

Aim and objectives: ***To evaluate the serum C3 as a predictor factor for NAFLD patients with diabetes and obesity.***

Patients and methods: ***This was prospective research carried out on seventy-five NAFLD patients attending the internal medicine department of Elsaied Galal University Hospital, with 25 healthy volunteers as a control. Cases were separated into four groups.***

Results: ***There was no significant distinction between the four groups regarding sex and age. There was a significant increase in WC and BMI in groups I, II, and IV; WC and BMI in group II were contrasted with group IV, and WC and BMI in group I were compared with group III. There was a significant increase in FBG, 2H-PPPG, TG, and LDL among groups II and III, C3 among groups II, III, and IV, and LDL among all groups. The greater the degree of C3, the greater the degree of BMI, FBG, ALT, TG, LDL, and U/S score among groups II, III, and IV. The lesser the degree of C3, the lesser the degree of AST among patients in group III.***

Conclusion: ***Serum complement C3 has shown promise as a biomarker for assessing therapeutic benefits and monitoring therapy outcomes in nonalcoholic fatty liver disease (NAFLD). It may also be useful for predicting how liver inflammation will get worse.***

Keywords: NAFLD; Complement C3; Diabetes Mellitus

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) ranges from mild steatosis to severe NASH. Patients with NAFLD, and especially NASH, are at increased risk for complications; the illness can develop into cirrhosis, HCC, and liver-related mortality due to fibrosis and other forms of liver damage. [1]

Until recently, the liver biopsy was the method of choice for assessing and determining the severity of fibrosis. However, this approach is not without its limitations, including sampling bias resulting from the small tissue sample, morbidity, post-procedure pain, observer variability, and mortality. [2]

Rensen et al. found that 74 percent of those with NAFLD showed evidence of complement system activation.[3] Serum complement C3a had a positive correlation with hepatic fat content among people at increased risk for type 2 diabetes as well as cardiovascular disease, by another study. [4]

According to a recent study, individuals with higher serum complement C3 levels are more likely to have NAFLD than those with lower C3 levels. [5]

This research aimed to assess serum C3 as a predictor factor for NAFLD patients with diabetes and obesity.

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2. Patients and methods

This prospective trial was conducted on seventy-five NAFLD cases, attending the internal medicine department at Elsaied Galal University Hospital, and twenty-five healthy volunteers as a control. Cases were separated into four groups. All patients were Egyptians; their ages ranged from 33 to 55. The study was performed from May 2022 to June 2023. patients were divided into four groups: Group I: 25 healthy volunteers as a control. Group II: 25 NAFLD individuals with diabetes and obesity. The diagnosis of NAFLD depends on standard clinical and ultrasound criteria. Group III: 25 NAFLD patients with diabetes only. Group IV: 25 NAFLD patients with obesity only.

Inclusion criteria: obese patients, diabetic patients, and NAFLD patients

Exclusion criteria: Patients with other causes of chronic liver diseases, e.g., HBV or HCV, autoimmune hepatitis, Wilson disease, and hemochromatosis. Patients with autoimmune connective tissue disorders. Cases with chronic kidney disease. People with coronary artery disease (CAD). Pregnant patients. Patients with malignancy. Hypertensive patients.

2.1.Methods:

All patients were subjected to the following:

Thorough history is taken, with special emphasis on age, sex, viral hepatitis, chronic illness, and family history of liver diseases. Full clinical examination, including (Clinical examination with special emphasis on abdominal discomfort at the right hypochondrium, jaundice, xanthomas). Anthropometric measures, Assessment of obesity: body mass index (BMI), and waist circumference (WC) in cm.

Lab investigations: 5 mL of blood samples were collected from all subjects for CBC using system XT-1500, FBG, 2-h-PPG (hexokinase method), AST, ALT, GGT, serum albumin, bilirubin, prothrombin time, prothrombin concentration, INR, serum creatinine, and serum lipid profile.

Assessment of serum C3 (using the ELISA kit): Standard Curve Range: 0.05–30 mg/mL Sensitivity: 0.025mg/ml, Size: 96 wells or 48 wells, Temperature range for reagent storage: two to eight degrees Celsius Keep it at -20 degrees Celsius for storage beyond the expiration date, which is six months. Refrain from performing multiple thawing cycles. After opening individual reagents, it is advised that the kit be utilized within one month.

2.2.Assay principle

This reagent contains an enzyme-linked immunosorbent assay (ELISA). Before use, the plate was coated with a human C3 antibody. The sample-bound C3 subsequently attaches to the antibodies coated in the wells. Following this, a human C3 antibody that has been biotinylated binds to C3 in the sample. Following the addition of streptavidin-HRP, it binds to the biotinylated C3 antibody. Unbound Streptavidin-HRP is eliminated during the washing phase after incubation. Following the addition of substrate solution, coloration occurs proportionally to the quantity of human C3. The introduction of a corrosive stop precipitates the end of the reaction.

2.3.Statistical analysis

The mean was used to analyze the results obtained and standard deviation, student t-test, standard error, linear correlation coefficient, chi-square, ROC curve, and analysis of variance (ANOVA) tests in SPSS software version 17. The tests used were the chi-square test, analysis of variance (ANOVA) tests, ROC curve, sensitivity, PPV, NPV, accuracy, and Pearson correlation coefficient (P value < 0.05 is considered statistically significant). A P value > 0.05 is considered statistically non-significant.

3. Results

Table 1. Comparison among the examined 4 groups as regards demographic data and medical history.

		GROUP I	GROUP II	GROUP III	GROUP IV	TEST VALUE	P-VALUE	SIG.
		No. = 25	No. = 25	No. = 25	No. = 25			
SEX	Female	14 (56.0%)	13 (52.0%)	11 (44.0%)	11 (44.0%)	1.080*	0.782	NS
	Male	11 (44.0%)	12 (48.0%)	14 (56.0%)	14 (56.0%)			
AGE	Mean ± SD	43.68 ± 5.38	42.40 ± 6.52	42.84 ± 7.00	44.68 ± 5.34	0.678•	0.567	NS
	Range	35 – 55	33 – 53	33 – 55	35 – 55			
MEDICAL HISTORY								
DM	No	25 (100.0%)	0 (0.0%)	0 (0.0%)	25 (100.0%)	100.000*	0.000	HS
	Yes	0 (0.0%)	25 (100.0%)	25 (100.0%)	0 (0.0%)			
HTN	No	25 (100.0%)	25 (100.0%)	25 (100.0%)	25 (100.0%)	-	-	-
	Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			

Student t test *p is significant at <0.05*: Chi-square test; •: One Way ANOVA test

This table demonstrated that there was no significant variation among the four groups with regard to sex and age. Table 1

Table 2. Comparison between groups I, II, III, and IV concerning anthropometric measurements.

ANTHROPOMETRIC MEASUREMENT		GROUP I (HEALTHY CONTROL)	GROUP II (OBESE DIABETIC)	GROUP III (DIABETIC NON-OBESE)	GROUP IV (OBESE NON-DIABETIC)	TEST VALUE	P-VALUE	SIG.
		No. = 25	No. = 25	No. = 25	No. = 25			
WC	Mean ± SD	81.24 ± 4.41	98.76 ± 8.16	90.72 ± 5.14	94.36 ± 5.07	41.015•	0.000	HS
	Range	74 – 88	89 – 123	83 – 101	86 – 105			
BMI	Mean ± SD	23.60 ± 1.22	34.48 ± 1.85	23.2 ± 1.8	31.08 ± 1.44	206.694•	0.000	HS
	Range	22 – 25	33 – 40	24 – 28	30 – 35			

POST HOC ANALYSIS

	Group I Vs group II	Group I Vs group III	Group I Vs group IV	Group II Vs group III	Group II Vs group IV	Group III Vs group IV
WC	0.003	0.000	0.701	0.000	0.009	0.000
BMI	0.000	0.000	0.258	0.000	0.000	0.000

One Way ANOVA test

There was a significant increase in WC and BMI in groups I, II, and IV; WC and BMI in group II in contrast to group Iv; and WC and BMI in group I compared with group III. Table 2

Table 3. Comparison among groups I, II, III, and IV concerning laboratory investigations.

LABORATORY INVESTIGATIONS		GROUP I (HEALTHY CONTROL)	GROUP II (OBESE DIABETIC)	GROUP III (DIABETIC NON-OBESE)	GROUP IV (OBESE NON-DIABETIC)	TEST VALUE	P-VALUE	SIG.
		No. = 25	No. = 25	No. = 25	No. = 25			
FFG	Mean ± SD	79.08 ± 6.19	172.00 ± 27.65	160.76 ± 16.92	102.32 ± 8.48	173.959•	0.000	HS
	Range	70 – 95	128 – 220	129 – 184	88 – 120			
2H-PPPG	Mean ± SD	160.44 ± 13.37	293.92 ± 34.80	280.20 ± 37.76	131.00 ± 5.63	240.043•	0.000	HS
	Range	120 – 178	240 – 390	220 – 380	122 – 140			
HB	Mean ± SD	10.82 ± 1.35	9.75 ± 0.71	11.00 ± 1.18	11.10 ± 1.14	7.678•	0.000	HS
	Range	8 – 13	8.8 – 11	8.9 – 13	8.9 – 14			
ALT	Mean ± SD	47.00 ± 3.91	46.80 ± 3.70	36.12 ± 4.90	46.40 ± 3.97	41.085•	0.000	HS
	Range	40 – 55	40 – 55	28 – 45	40 – 55			
AST	Mean ± SD	44.48 ± 3.11	44.36 ± 3.28	32.12 ± 5.29	44.24 ± 3.31	63.088•	0.000	HS
	Range	40 – 50	40 – 50	25 – 45	40 – 50			
SERUM C3	Mean ± SD	98.56 ± 18.25	320.68 ± 4.05	220.56 ± 2.45	272.68 ± 3.85	2466.099•	0.000	HS
	Range	75 – 135	315 – 327	215 – 225	266 – 280			
S.CREAT	Mean ± SD	1.06 ± 0.17	1.06 ± 0.17	1.06 ± 0.17	1.06 ± 0.17	0.000•	1.000	NS
	Range	0.8 – 1.3	0.8 – 1.3	0.8 – 1.3	0.8 – 1.3			
INR	Mean ± SD	1.04 ± 0.14	1.04 ± 0.14	1.04 ± 0.14	1.04 ± 0.14	0.000•	1.000	NS
	Range	0.8 – 1.3	0.8 – 1.3	0.8 – 1.3	0.8 – 1.3			
TG MG/DL	Mean ± SD	134.00 ± 12.33	240.60 ± 27.05	182.76 ± 19.12	240.60 ± 27.05	133.723•	0.000	HS
	Range	110 – 150	200 – 300	145 – 217	200 – 300			
LDL	Mean ± SD	110.58 ± 15.51	145.80 ± 18.06	158.85 ± 23.15	145.80 ± 18.06	30.059•	0.000	HS
	Range	83 – 136	95 – 176	124 – 200	95 – 176			
HDL	Mean ± SD	48.64 ± 3.66	39.56 ± 3.70	36.32 ± 3.05	44.2 ± 2.6	80.152•	0.000	HS
	Range	43 – 55	33 – 47	31 – 42	40– 46			

POST HOC ANALYSIS

	Group I Vs group II	Group I Vs group III	Group I Vs group IV	Group II Vs group III	Group II Vs group IV	Group III Vs group IV
FBG	0.000	0.000	0.000	0.022	0.000	0.000
2H-PPG	0.000	0.000	0.000	0.072	0.000	0.000
HB	0.001	0.572	0.387	0.000	0.000	0.763
ALT	0.865	0.000	0.610	0.000	0.734	0.000
AST	0.913	0.000	0.826	0.000	0.913	0.000
SERUM C3	0.000	0.000	0.000	0.000	0.000	0.000
TG MG/DL	0.000	0.000	0.000	0.000	1.000	0.000

LDL	0.000	0.000	0.000	0.016	1.000	0.016
HDL	0.000	0.002	0.000	0.000	1.000	0.000

There was a significant increase in FBG, 2H-PPPG, TG, and LDL among groups II and III, C3 among groups II, III, and IV, and LDL among all groups. Table 3

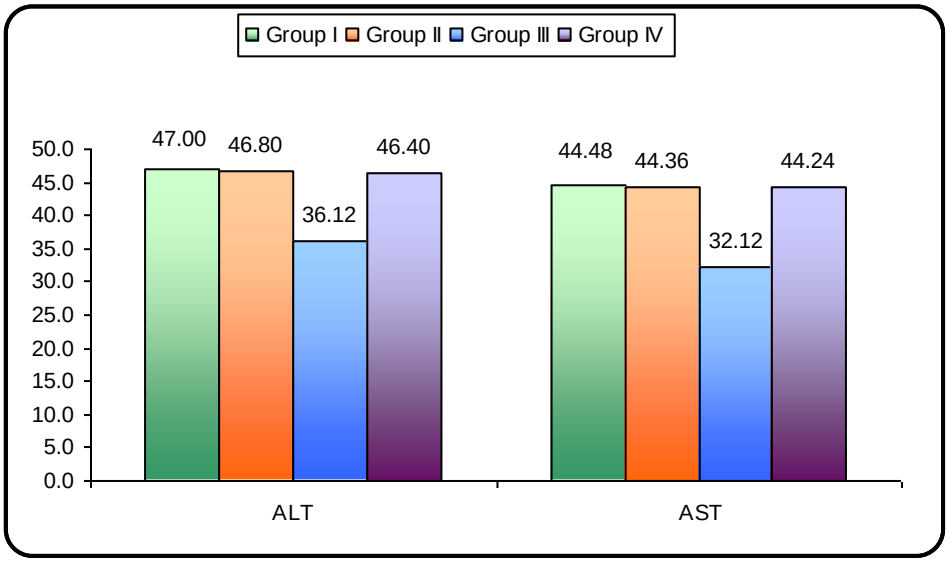

Figure 1. Comparison between groups I, II, III, and IV regarding ALT and AST.

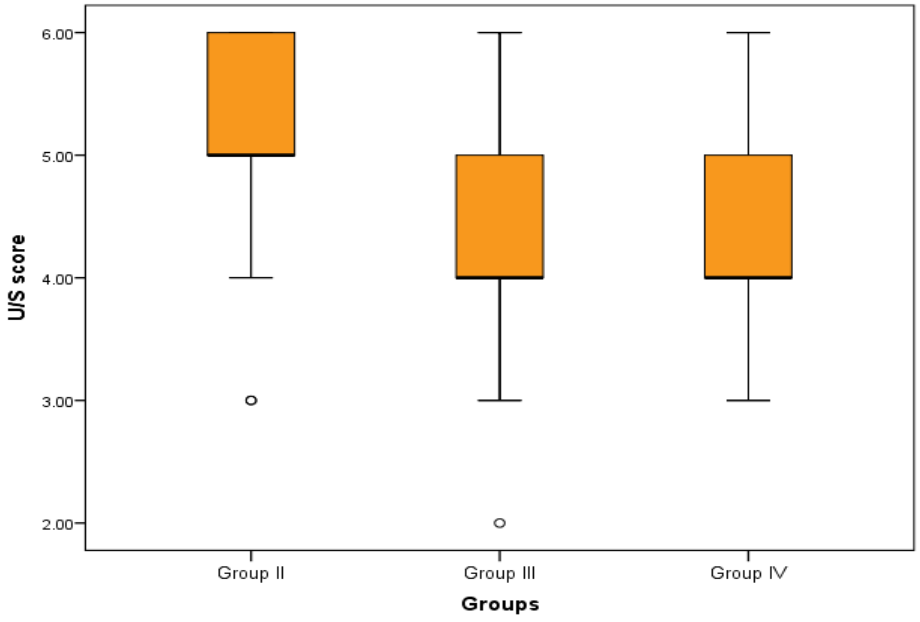

Figure 2. Comparison between groups II, III, and IV regarding the U/S score.

Table 4. Correlation of Serum C3 with Other Studied Parameters in Group II

GROUP II	SERUM C3	
	R	P-value
AGE	0.224	0.281
WC	-0.115	0.584
BMI	0.437*	0.029
FBG	0.498*	0.011
2H-PPPG	-0.314	0.127
HB (ADD)	-0.279	0.177
ALT	0.485*	0.014
AST	0.262	0.206
S.CREAT	0.132	0.528
INR	0.080	0.703
TG MG/DL	0.708**	0.000
LDL	0.527**	0.007
HDL	0.018	0.934
U/S SCORE	0.560**	0.004

Spearman correlation coefficient

There were positive correlations between C3, BMI, FBG, ALT, TG, LDL, and U/S score among group II. The greater the degree of C3, the greater the degree of BMI, FBG, ALT, TG, LDL, and U/S score among group II. (Table 4)

Table 5. Correlation of Serum C3 with Other Studied Parameters in Group III

GROUP III	SERUM C3

	R	P-value
AGE	0.051	0.810
WC	-0.215	0.303
BMI	-0.078	0.711
FBG	0.718**	0.000
2H-PPPG	0.301	0.143
HB (ADD)	0.114	0.413
ALT	0.004	0.984
AST	-0.485*	0.014
S.CREAT	-0.051	0.808
INR	-0.028	0.894
TG MG/DL	0.406*	0.044
LDL	-0.066	0.755
HDL	-0.029	0.889
U/S SCORE	0.090	0.669

Spearman correlation coefficient

There was a significant positive correlation between C3, FBG, and TG, i.e., the greater the degree of C3, the greater the degree of FBG and TG among patients in group III. But there is a significant correlation between C3 and AST, i.e., the lesser the degree of C3, the lesser the degree of AST among patients in group III. (Table 5)

Table 6. Correlation of Serum C3 with Other Studied Parameters in Group IV

GROUP IV	SERUM C3	
	R	P-value
AGE	-0.141	0.503
WC	-0.406*	0.044
BMI	-0.312	0.129
FFG	0.117	0.576
2H-PPPG	0.166	0.428
HB	0.233	0.262
ALT	0.506**	0.010
AST	-0.009	0.966
S.CREAT	-0.251	0.227
INR	0.319	0.120
TG MG/DL	0.480*	0.015
LDL	0.430*	0.032
HDL	-0.240	0.247
U/S SCORE	0.423*	0.035

Spearman correlation coefficient

There was a significant negative correlation between C3 and WC; the lesser the degree of C3, the lesser the degree of WC among patients in group IV, but there was a significant positive correlation between C3, ALT, TG, LDL, and U/S score; the greater the degree of C3, the greater the degree of ALT, TG, LDL, and U/S score among patients in group IV. (Table 6)

4. Discussion

In terms of sex and age, there was not a significant distinction among the four groups.

Rosato et al.[6] suggested that CKD is more common in those with NAFLD, occurring in between twenty percent and fifty percent of those with NAFLD, and this may hasten the onset and progression of CKD. The severity of NAFLD was found to be directly connected with CKD in a meta-analysis study that evaluated the incidence and prevalence of CKD in people with simple fatty liver, NASH, and advanced fibrosis. In contrast, our findings showed no significant variations in serum creatinine levels between the groups.

According to our findings, serum C3 levels varied significantly between groups. This study showed that the levels of C3 in NAFLD patients were much higher than in healthy controls and that C3 activity was linked with disease severity. It also showed that the levels of C3 are extremely high in NAFLD patients who have both diabetes and obesity, as opposed to just one of these conditions.

In agreement with our results, the Xu, Chen, et al.[4] cross-sectional study revealed that the prevalence and severity of NAFLD are both highly associated with serum complement C3 levels in the Chinese population.

The current research revealed that complement C3 was significantly higher in obese cases, either with or without diabetes, and diabetic-non-obese patients compared with lean, healthy subjects. BMI and NAFLD are both associated with elevated serum C3 levels.

Also, Ragab et al.[7] noticed that the average BMI of NAFLD cases was 32 ±7.7 kg/m2, while that of healthy controls was 23.8 ±1.5 kg/m2.

Our study's findings indicated that there was a significant variation in the mean waist circumference and the mean BMI among the four groups.

This is in agreement with Himoto et al.[8] who found that since the levels of complement C3 had a substantial correlation with body mass index (BMI) and increased with the progression of liver steatosis and liver fibrosis, the researchers concluded that complement C3 levels might be an indicator of obesity and hepatic steatosis.

Regarding laboratory investigations, our results showed a statistically significant disparity between the groups in FBG and 2H-PPG, with a P-value < 0.01.

In agreement with us, Borné et al.[9] found that although complement C3 is associated with diabetes incidence, there is no evidence to suggest a causal relationship.

Our results revealed significant variation among the four liver function tests (ALT and AST) groups.

Feng et al.[10] also found that the presence of nonalcoholic fatty liver disease (NAFLD) increased with increasing complement C3 levels, and complement C3 was a risk factor for the existence of NAFLD.

Regarding HB level, there was a significant distinction among groups, with a P-value < 0.01.

Opposing our finding, Ragab et al.[7] (studied groups) found no significant variations between NAFLD individuals and healthy controls in terms of RBC count, hemoglobin level, or platelet count

(P > 0.05). Although the two groups had similar mean values for WBCs, there was a significant distinction (P = 0.038) among the groups.

Our results showed a significant disparity among Groups I, II, III, and IV in TG, LDL, and HDL lipid profiles, with a P-value < 0.01.

Zhong et al.[11] found that by controlling the formation of tRNA-derived fragments (glycine tRNA-derived fragments) in NAFLD, complement C3 enhanced TG accumulation and contributed to liver damage and steatosis.

Our results showed a significant disparity amongst four groups in U/S score (including Fib-4 score and NFS). The range in Group II was 3–6, the range in Group III was 2–6, and the range in Group IV was 3–6.

Angulo et al.[12] said that although pathological investigation is still the gold standard for diagnosing diseases, ultrasound-based detection of nonalcoholic fatty liver disease (NAFLD) is frequently utilized in clinical settings as a noninvasive, cost-effective screening method with a sensitivity of 89 percent and a specificity of 93 percent. It would be very interesting to see more studies on the correlation between serum C3 levels and the histological severity of NAFLD.

5. *Conclusion*

Serum complement C3 concentrations increased substantially among individuals with NAFLD and those with a worsening NAFLD fibrosis score. As a result, measuring serum complement C3 may be a useful method for predicting the progression of liver inflammation, providing a possible biomarker for evaluating the efficacy of treatments and monitoring the results of NAFLD treatments..

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article

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Conflicts of interest

There are no conflicts of interest.

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