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ORIGINAL ARTICLE

Altered retinol binding protein-4 (RBP-4) mRNA and serum levels in obesity are associated with the susceptibility and progression of Non-alcoholic fatty liver disease (NAFLD)

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is a rapidly progressive disease and, nowadays, is the main cause of chronic liver disease in children and adults. This study aimed to investigate serum and mRNA levels of retinol-binding protein-4 (RBP-4) in obese Egyptian children vs adults and to assess its correlation with susceptibility and progression of NAFLD.

Methods: The study comprised 50 obese patients (25 adults and 25 children) and 50 healthy controls. All participants were subjected to full clinical, anthropometric, and biochemical assessment. Liver steatosis was assessed by the controlled attenuation parameter component. Fatty liver indexes and hepatic steatosis index were calculated. RBP-4 mRNA and serum levels were tested.

Results: Our results revealed significantly higher values of serum RBP-4 and its mRNA levels in obese groups compared to control groups. In obese pediatric patients, the serum RBP-4 and its mRNA levels were elevated in the NAFLD patients (75.1±4.8 and 6.2±4.5, respectively) in comparison to the non-NAFLD group (56.2±1.9 and 4.6±0.14, respectively), P <0 .001. Additionally, in the obese adult group, the serum RBP-4 and its mRNA values were significantly higher in the NAFLD obese group (75.5±3.5 and 6.3±3.7, respectively) in comparison to the non-NAFLD group (56.5±15.4, and 4.2±0.13, respectively). Obesity indices and hepatic and metabolic dysfunction parameters were significantly positively correlated with serum RBP-4 and its mRNA values in obese groups.

Conclusion: RBP-4 mRNA and serum levels are higher in obese patients compared to controls, particularly in NAFLD patients. RBP-4 mRNA and serum RBP-4 could be non-invasive biomarkers of NAFLD.

Keywords: Non-alcoholicNon-alcoholic fatty liver disease**,** fatty liver indexes, obesity, retinol binding protein-4, controlled attenuation parameter.

INTRODUCTION

Non-alcoholicNon-alcoholic fatty liver disease (NAFLD) is a chronic progressive disease. Compelling evidence suggests that NAFLD is the most prevalent hepatic disease in all age groups [1]. The prevalence of NAFLD is currently estimated at up to 90% in morbidly obese adults [2]and about 34.2% among youth with obesity [3]. It is estimated by 2030, NAFLD will become the major cause of liver transplantation [4].

Accumulating studies have reported that the variety of NAFLD ranges from steatohepatitis to fibrosis [5]. Recently published studies highlighted the complexity of the pathogenesis of

NAFLD. Obesity is still the leading cause of NAFLD, and its severity through immune dysregulation [6,7].

Importantly, it was noted that the severe form of NAFLD, such as fibrosis and cirrhosis, was common in children due to the increased prevalence of obesity in this age group [8].

Various clinical studies prove that dysregulations of adipocytokine patterns contribute to adipose tissue dysfunction [9]. Adipose tissue secretes many adipokines, including retinol-binding protein-4 (RBP-4), which leads to many diseases such as NAFLD. RBP-4 is expressed in the liver and adipose tissue, and if it is overexpressed, it increases oxidative stress and mitochondrial dysfunction [10].

In previous Egyptian study conducted on young adults found a high prevalence of steatosis, about 30%, and fibrosis 5% [1]. Current evidence confirmed the lack of diagnostic and prognostic methods for NAFLD. Therefore, the objective of this study was to investigate the underlying molecular mechanisms governing the pathogenicity of it [4]. To the best of our knowledge, this is the first study to investigate mRNA of retinol-binding protein-4 (RBP-4) and its serum level in obese Egyptian children vs adults and to assess its correlation with susceptibility and progression of NAFLD.

METHODS

This case-control study enrolled 50 obese patients (25 adults and 25 children) and 50 healthy non-obese controls (25 adults and 25 children). The flowchart of this study is presented in supplementary figure 1 . Analyses were operated according to operating methods in Zagazig University Hospital. The assessment of serum RBP4 was investigated by ELISA.

Ethics approval and consent to participate: Written informed consent was obtained from each participant to use the clinical and biochemical data for research. In patients aged < 18 years, the research was accepted by the research ethical committee of the Faculty of Medicine, Zagazig University (IRB≠, 461/23- JUNE-2024).

RNA Isolation and qRT-PCR

Total RNA was taken from the peripheral blood mononuclear cells (PBMCs) with TRIzol reagent (Invitrogen, California, USA) according to the manufacturer's guidelines. The primer sequences are shown in. GAPDH worked as a reference. The primers were as follows.

Gene	Forward (5'-3:)	Reverse primer (5'-3')
RBP-4 mRNA	5′-TCTGCCTAGAGAGGCAGTACA-3′	5′-AACTGTTTCTTGAGGGTCTGCT-3′
GAPDH	5′-TGGGGAAGGTGAAGGTCGGA-3′	5′-GGGATCTCGCTGCTCGAAGA-3′

STATISTICAL ANALYSIS

The study's results were done using SPSS version 26 (Version 26.0. Armonk, NY: IBM Corp). Descriptive summary statistics were calculated and presented as mean ± SD. We performed the t-test, the Mann-Whitney U Test. Additionally, we used the following tests to explore the findings: Pearson correlation, linear regression, and ROC curve tests were done. $p < 0.05$ was considered significant.

RESULTS

Out of 50 patients (25 children and 25 adults) referred to our tertiary center with obesity, only 26 patients had NAFLD (7 children and 19 adults) as shown in the flowchart, figure 1.

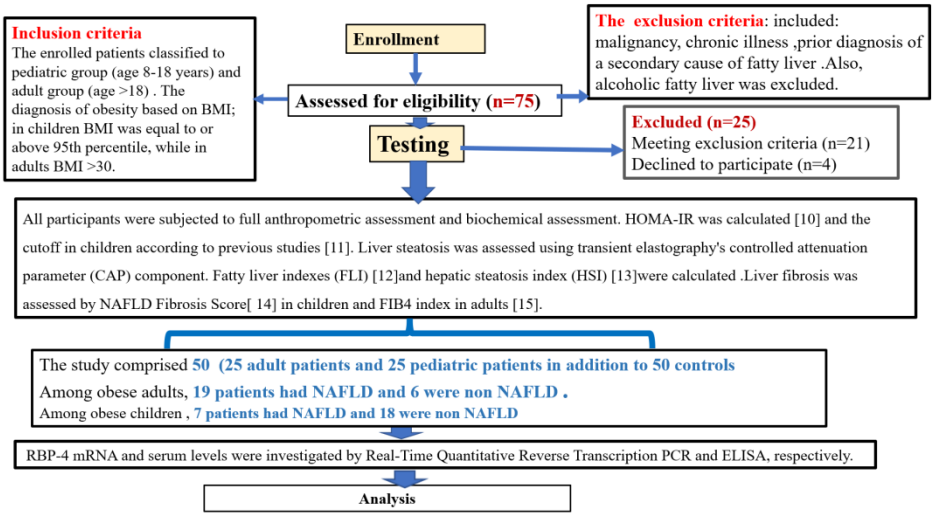

Figure 1: flowchart of the study

Clinical characteristics of obese children according to NAFLD status are presented in Table 1. Concerning the anthropometric indices of obesity, patients in the NAFLD group had higher WC and BMI compared to other groups. Additionally, among lipid profile parameters, only TC and TG were elevated in NAFLD among obese children. Regarding glycemic profile, HbA1c, FPG, FSI, and HOMA-IR were elevated in NAFLD children. As expected, the laboratory and radiology, as well as calculated indices of steatosis, were increased in the NAFLD group compared to a non-NAFLD group such as ALT, GGT, alkaline phosphatase, fatty liver index (FLI), hepatic steatosis index (HSI), pediatric NAFLD (PNFS), controlled attenuation parameter(CAP) and fibrosis stages according to the results of transient elastography, P<0.001, table 1.

Regarding the adult obese group, regarding the anthropometric indices of obesity, patients in the NAFLD group had higher WC and BMI compared to another group. Furthermore, among glycemic profile tests, HbA1c, FPG, and HOMA-IR were elevated in the NAFLD group. As expected, the laboratory and radiology, as well as calculated indices of steatosis, were increased in the NAFLD group compared to a non-NAFLD group such as ALT, alkaline phosphatase, FLI, HSI, CAP, and fibrosis stages according to the results of transient elastography. P<0.001, table 2

To assess serum RBP-4 levels, we applied the ANOVA test, and the results are presented in Figure 2. Interestingly, we detected significant differences between the studied groups. Among children, the NAFLD group (75.1±4.8) had higher values compared to the non-NAFLD group (56.2±1.9). Additionally, The results of adult group testing showed that the NAFLD group (75.5±3.5) had higher values as compared to non-NAFLD (56.5±15.4), figure 2, P <0 .001.

To determine whether these findings were in harmony with the results of RBP-4 mRNA, we performed an analysis of our PCR findings, and we found significant differences between the studied groups. Among children, the NAFLD group (6.2±4.5) had higher values compared to the non-NAFLD group (4.6±0.14). Additionally, The results of adult group testing showed that the NAFLD group (6.3±3.7) had higher values as compared to non-NAFLD (4.2±0.13), figure 2, P <0 .001.

To elucidate the associations between studied variables and RBP-4 mRNA and serum levels, We have confirmed that by applying Pearson correlation, age, WC, BMI, TC HbA1c, FPG, FSI, HOMA-IR, ALT, Alkaline phosphatase and HSI values were significantly positively correlated with serum RBP-4 and its mRNA values in obese groups as described in table 3, P <0 .001.

According to the results of correlations, there were many variables significantly correlated to both markers; thus, we therefore examined the independent factors that were associated with RBP-4 mRNA and serum levels in the prediction of NAFLD that only ALT, HIS and CAP were the independent factors that were associated with serum RBP-4, table 4, P <0 .001. Regarding RBP-4 mRNA, the independent factors that were associated with it were ALT and CAP, table 4, P <0 .001.

To discover the efficiency of RBP-4 mRNA and serum RBP-4 in comparison to other studied variables in predicting obesity among the studied group. We applied ROC curve analysis, and the results are described in Figure 3a and the supplementary file, Table S1.

Additionally, the efficiency of RBP-4 mRNA and serum RBP-4 in comparison to other studied variables in differentiation of children with NAFLD among obese children should be detected. The results of the ROC curve analysis are explained in Figure 3b and in the supplementary file, Table S2.

To examine the effectiveness of RBP-4 mRNA and serum RBP-4 in comparison to other studied variables in the differentiation of adults with NAFLD among obese adults. The results of the ROC curve analysis are explained in Figure 3c and in the supplementary file, Table S3. Based on ROC curve results, RBP-4 mRNA and serum RBP-4 had higher sensitivity and specificity in the determination of obesity and NAFLD among children compared to other studied variables. Meanwhile, among adults, the sensitivity and specificity of both RBP-4 mRNA and serum RBP-4werepoor.

Table 1: Clinical characteristics of obese children according to NAFLD status

Parameters	Non-NAFLD N=18	NAFLD N=7	P value
Age (year)	11.9±2.3	13.18±1.4	0.543
Sex, n(%) Female, n(%) Male, n(%)	 7(38.9%) 11(61.1%)	 3(42.9%) 4(57.1%)	0.855
WC, cm	97.3±2.5	108.3±5.6	<0.001*
BMI	30.1±4.3	38.4±8.5	<0.001*
BMI Z-score	2.1±0.4	2.58±0.9	0.07
TC (mg/dL)	202.5± 5.7	217.85±16.1	<0.05*
TG (mg/dL)	213.5± 7.8	239.8±22.2	<0.001*
LDL (mg/dL)	174.5± 5.2	175.1±9.1	0.841
HDL (mg/dL)	39.5±1.5	36.7±4.1	0.078
HbA1c	5.95±0.9	6.64±1.1	<0.05*
FPG (mg/dL)	89.1±11.4	119.5± 35.3	<0.001*
FSI (lU/mL)	22.1±2.9	30.42±12.9	<0.05*
HOMA-IR	5.1±1.7	9.12±3.5	<0.05*
AST(IU/L)	55.7±13.15	54.6±12.4	0.843
ALT (IU/L)	69.2±3.7	84.8±11.3	<0.001*
GGT (IU/L)	60.7±8.15	63.76±11.4	<0.05*
Alkaline phosphatase (IU/L)	169.2± 1.76	392.2± 52.1	<0.001*
Platelet(cell×10^3/µl)	180.3±54.5	183.5±34.7	0.840
Albumin (g/dl)	3.8±0.1	3.8±0.3	0.805
FLI Normal Intermediate Severe	 18(100%) - -	 - 5 2	<0.001*
Hepatic steatosis index (HSI)	31.2±1.76	50. 1±4.8	<0.001*
CAP (dB/m) mean± SD **S1 n, %** **S2** **S3**	165.4± 14.6 0 0 0	282.5± 34 .3 3 2 2	<0.001*
PNFS	.4635 .11923	.6552 .27207	<0.001*
Fibrosis F0-F1 F2 F3 F4	 - - -	 4 2 1 0	<0.001*
Serum RBP4 (ug/ml)	30.20±5.94	59.88±13.92	[3]0.001*

WC, waist circumference; BMI, body mass index; FPG, fasting plasma glucose; FSI, fasting serum insulin, HOMA-IR, homeostasis model assessments of insulin resistance, AST; aspartate aminotransferase, ALT; alanine aminotransferase, GGTP; gamma-glutamyltranspeptidase; FLI; fatty liver index, HSI; hepatic

steatosis index, PNFS; Pediatric NAFLD Fibrosis Score, , CAP ; controlled attenuation parameter.*P < 0.05 when compared with control group

Table 2: Clinical characteristics of obese adults according to NAFLD status

Parameters	Non-NAFLD N=6	NAFLD N=19	P value
Age (year)	40.6±3.8	38.9±2.7	0.177
Sex, n(%) Female, n(%) Male, n(%)	 3(50%) 3(50%)	 9(47.3%) 10(52.7%)	0.910
WC, cm	102.3±15.5	122.5 ±12.6	<0.001*
BMI	35.6±2.8	41.1±7.4	<0.001*
TC (mg/dL)	202.3± 5.5	207.6± 6.1	0.293
TG (mg/dL)	213.3± 5.5	218.6± 6.1	0.322
LDL (mg/dL)	196.6± 2.8	196.7± 3.8	0.992
HDL (mg/dL)	40.5±1.1	40.2±1.1	0.905
HbA1c	6.6±0.8	7.9±1.06	<0.001*
FPG (mg/dL)	124.1± 39.2	154.5± 29.3	<0.001*
FSI (lU/mL)	31.6±9.91	35.2±6.6	0.147
HOMA-IR	10.4±6.04	13.7±4.17	<0.001*
AST(IU/L)	54.6±0.847	55.2±1.83	0.536
ALT (IU/L)	64.5±13.13	88.1±5.1	<0.001*
GGT (IU/L)	59.6±0.84	60.2±1.8	0.707
Alkaline phosphatase (IU/L)	175.1± 11.7	379.2± 52.7	<0.001*
Platelet(cell×10^3/µl)	179.6±0.84	180.2± 1.8	0.971
Albumin (g/dl)	3.8±0.17	3.7±0.32	0.179
Fatty liver index (FLI) Normal Intermediate Severe	6 0 0	0 11 8	<0.001*
Hepatic steatosis index (HSI)	37.8±0.87	50.5±3.5	<0.001*
CAP (Db/m) **S1** **S2** **S3**	 0 0 0	 4 7 8	<0.001*
Fibrosis F0-F1 F2 F3 F4	 6 0 0 0	 8 7 4 0	<0.001*

WC, waist circumference; BMI, body mass index; FPG, fasting plasma glucose; FSI, fasting serum insulin, HOMA-IR, homeostasis model assessments of insulin resistance, AST; aspartate aminotransferase, ALT; alanine aminotransferase, GGTP; gamma-glutamyltranspeptidase; FLI; fatty liver index, HSI; hepatic steatosis index, PNFS; Pediatric NAFLD Fibrosis Score, FIB4; fibrosis-4 score, CAP; controlled attenuation parameter.*P < 0.05 when compared with control group.

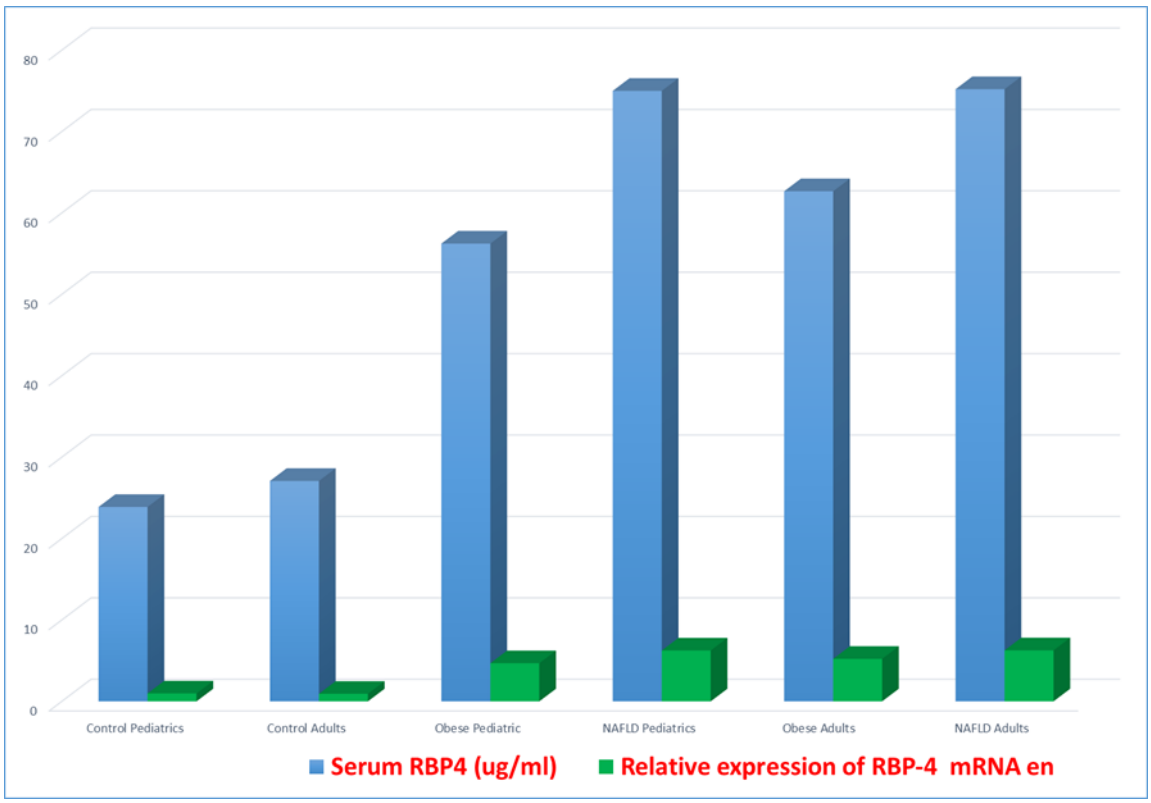

Figure 2: Comparison of RBP-4 mRNA and serum levels among studied participants

Table 3: Pearson correlation of RBP-4 mRNA and serum levels with clinical, anthropometric, and biochemical characteristics in obese patients.

Variables	Serum RBP4 (ug/ml)		RBP-4 mRNA	
	r	p		
Age	0.444	<0.001*	0.543	<0.001*
WC	0.635	<0.001*	0.546	<0.001*
BMI	0.610	<0.001*	0.511	<0.001*
TC	0.317	<0.001*	0.336	<0.001*
TG	0.024	0.893	0.198	0.0120
LDL	0.158	0.280	0.223	0.119
HDL	-0.055	0.704	-0.034	0.754
HbA1c	0.538	<0.001*	0.609	<0.001*
FPG	0.497	<0.001*	0.522	<0.001*
FSI	0.545	<0.001*	0.611	<0.001*
HOMA-IR	0.375	<0.01*	0.354	<0.001*
AST	0.131	0.452	0.193	0.179
ALT	0.567	<0.001*	0.434	<0.001*
GGT	0.692	<0.001*	0.602	<0.001*
Alkaline phosphatase	0.587	<0.001*	0.651	<0.001*
Platelet	0.133	0.356	0.222	0.121
Albumin	-0.039	0.787	-0.250	0.080
Hepatic steatosis index	0.852	<0.001*	0.765	<0.001*

WC, waist circumference; BMI, body mass index; FPG, fasting plasma glucose; FSI, fasting serum insulin, HOMA-IR, homeostasis model assessments of insulin resistance, AST; aspartate aminotransferase, ALT; alanine aminotransferase, GGTP; gamma-glutamyltranspeptidase; *P < 0.05

Table 4: Linear regression analyses in obese patients to test the influence of the main independent variables against RBP-4 mRNA and serum levels

{Model}		Unstandardized Coefficients		Standardized Coefficients	t	P value	95% C.I.	
		B	Std. Error	Beta			Lower Bound	Upper Bound
Serum RBP-4	(Constant)	2.004	4.326		0.463	0.644	-6.585	10.594
	BMI	0.126	0.147	0.044	0.855	0.394	-0.167	0.419
	ALT	0.551	0.048	0.745	11.406	<0.001*	0.455	0.647
	HSI	0.071	0.002	0.886	29.704	<0.001*	0.066	0.075
	CAP	0.062	0.010	0.220	6.043	<0.001*	0.041	0.082
	HOMA-IR	0.006	0.004	0.020	1.579	0.118	-0.002	0.014
RBP-4 mRNA	(Constant)	1.520	4.403		0.345	0.731	-7.223	10.263
	BMI	0.125	0.148	0.044	0.847	0.399	-0.168	0.419
	ALT	0.557	0.049	0.754	11.290	<0.001*	0.459	0.655
	HSI	0.047	0.080	0.016	0.584	0.561	-0.113	0.206
	CAP	0.064	0.011	0.228	5.921	<0.001*	0.042	0.085
	HOMA-IR	-0.078	0.120	-0.020	-0.651	0.516	0317	0.161

BMI, body mass index; ALT; alanine aminotransferase, HOMA-IR, homeostasis model assessments of insulin resistance HIS; hepatic steatosis index, CAP: controlled attenuation parameter, P < 0.05

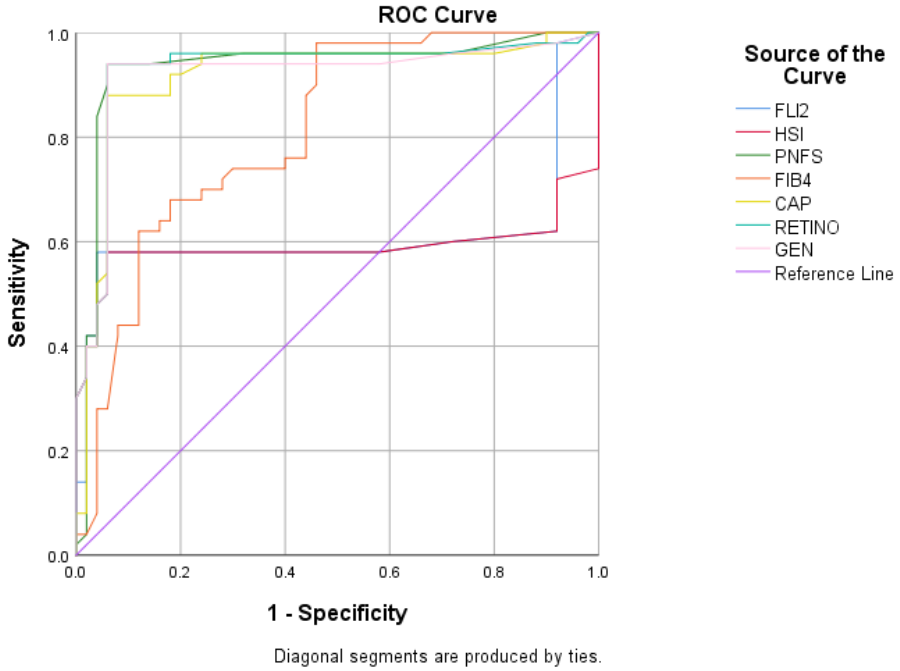

Figure 3a: ROC curve results of different variables to assess their diagnostic power in the differentiation of obese patients among studied variables.

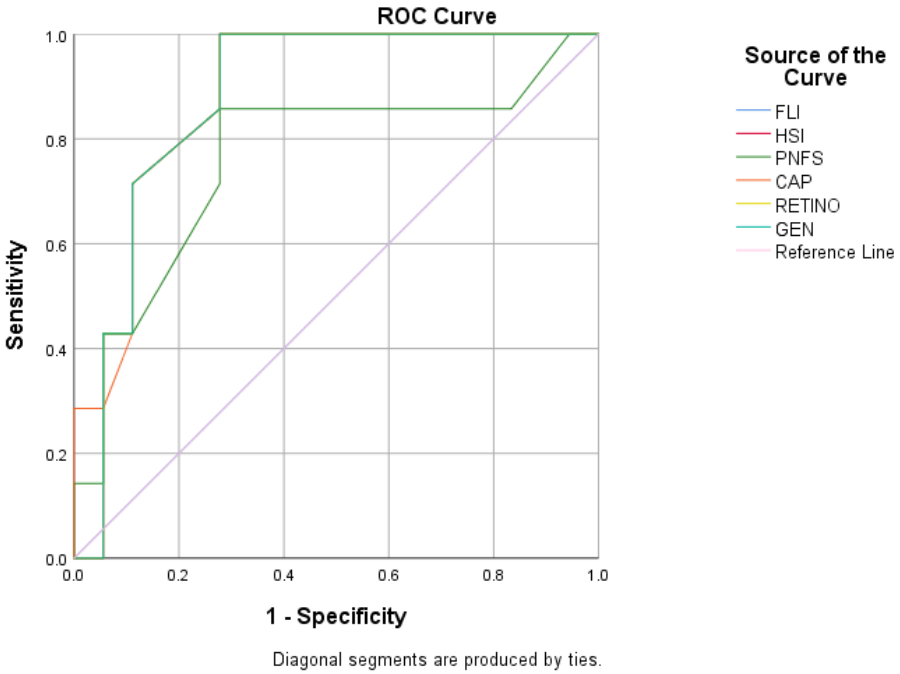

Figure 3b: ROC curve results of different variables to assess their diagnostic power in differentiation of children with NAFLD among obese children

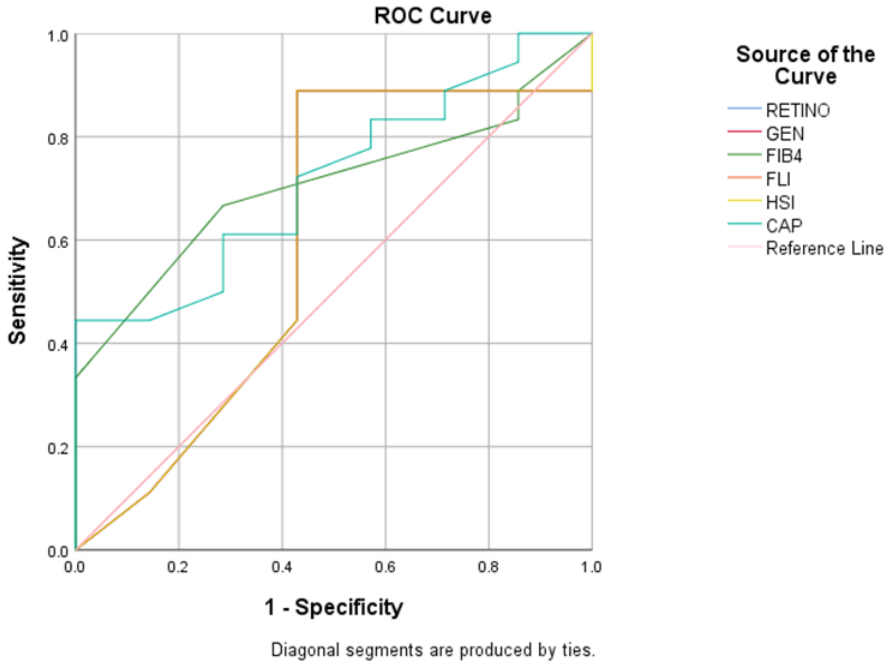

Figure 3c: Comparison of ROC curve results of different variables to assess their diagnostic power in differentiation of adults with NAFLD among obese adults

DISCUSSION

We investigated 50 obese patients, 25 children, and 25 adults. It is well known that liver biopsy is the gold standard for diagnosing NAFLD but is not indicated in all patients with suspected disease. It is invasive and expensive; thus, we used other non-invasive tests, as we mentioned in the flow chart. Our analysis indicated that out of 25 obese children, seven children (28 %) had NAFLD, and among 25 enrolled obese adults, 19 adults (73.1 %) had NAFLD.

It has been well recognized that obesity has a unique role in the pathogenesis of NAFLD, thus screening is recommended for obese and even overweight subjects with other risk factors such as insulin resistance [16]. In this regard, the prevalence of NAFLD varies according to the degree of obesity [17].

Similar to our result, a study conducted by Younossi et al. detected that the prevalence of NAFLD in the Middle East is about 25.24% of global NAFLD prevalence [17]. In the United States, NAFLD is account for 38% in obese children [18].

It has been postulated that NAFLD causes more load on world supplies. However, till now the awareness of this problem is less than expected [17]. Even more importantly, the pathogenesis of NAFLD remains to be explained. Thus, we performed this research to investigate cytokine dysregulations such as RBP -4 mRNA and its serum concentrations in obese children and adults to evaluate their ability to predict NAFLD in correlation with metabolic dysfunction as well as hepatic steatosis indices and markers.

Importantly, our analysis indicated that among obese children, the NAFLD kids had increased levels of RBP -4 mRNA and its serum in comparison to NAFLD kids. Furthermore, the findings of adult group testing showed that the NAFLD group had increased values compared to other subjects.

In agreement with our results, Yang et al. found higher values of RBP-4 in obesity, especially those with insulin resistance [19]. Additionally, Zovich and his colleagues detected higher expression of RBP-4 in adipose tissue [20]. Intriguingly, similar results were detected in a study conducted by Seo et al.; they found higher values of RBP -4 in NAFLD [21].

Noteworthy, the current results observed significantly higher levels of serum RBP-4 in obese children compared to control, and these results were in concordance with other studies conducted in Turkey [22]and China [23].

Despite the growing evidence that obesity and insulin resistance contributed to NAFLD [21], the study conducted by Nobili et al found that serum RBP4 levels decreased in NAFLD in particularly sever form [24]. These contradictory results seem to be due to the difference in the study population and sample size of the studied population.

To further evaluate our results, we tested the associations between serum RBP-4 and its mRNA and other studied parameters, and we detected that age, WC, BMI, TC, HbA1c, FPG, FSI, HOMA-IR, ALT, Alkaline phosphatase and HSI values were significantly positively correlated with serum RBP-4 and its mRNA values in obese groups. Taken together, these data show a firm correlation between dyslipidemia and hyperglycemia as well as parameters of steatosis. Furthermore, ALT, HIS, and CAP were the independent factors that were associated with serum RBP-4. Meanwhile, ALT and CAP were the independent factors associated with RBP-4 mRNA. It was previously reported that obesity is associated with dyslipidemia and hyperglycemia, leading to mitochondrial dysfunction, increased hepatic steatosis, and increased RBP4 mRNA expression [10].

To assess the efficacy of serum RBP4 and mRNA in the determination of obesity and NAFLD among obese children and adults in comparison to other studies markers such as FLI, HIS, CAP, and PNFS in children and FLI, HIS, CAP as well as FIB-4 INDEX in adults, we applied ROC curve and the results showed that RBP-4 mRNA and serum RBP-4 had higher sensitivity and specificity in the determination of obesity from control and NAFLD among children compared to other studied variables. Meanwhile, among adults, the sensitivity and specificity of both RBP-4 mRNA and serum RBP-4 were poor. The difference between adults and children could be related to the fact that the number of NAFLD in adults (n=19) was higher than in children (n=7).

Study limitations and strengths

The current study was the first to explore RBP-4 mRNA and its serum RBP-4 in both obese children and adults and to correlate their levels with hepatic and metabolic dysfunction parameters, particularly NAFLD. There were a few limitations of this study that must be considered when interpreting its findings. Firstly, the small sample size. Secondly, all participants are from a single center, and lastly, we depend on non-invasive methods for the assessment of NAFLD. There is a need for further large-scale multicenter longitudinal studies to confirm our results in this susceptible population.

CONCLUSION

we reported that RBP-4 mRNA and serum RBP-4 were higher in obese patients compared to controls, particularly in NAFLD patients. Furthermore, they showed a positive correlation with hepatic and metabolic dysfunction markers. We hypothesize that RBP-4 mRNA and serum RBP-4 could be non-invasive biomarkers of NAFLD. Further research is needed to assess its potential therapeutic significance.

Conflict of Interest: There are no financial conflicts of interest to disclose

Financial Disclosures: NA

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Table S1: Comparison of ROC curve results of different variables to assess their diagnostic power in differentiation of obese patients among studied variables.

Variables	AUC	Cutoff value	Sensitivity	Specificity	P value	95% C.I	
						lower	upper
Serum RBP4	0.929	43.5	94%	88%	<0.001*	0.869	0.989
RBP4 mRNA	0.919	3.2	93%	85%	<0.001*	0.855	0.983
FLI	0.608	21.4	50.3%	58.1%	<0.001*	0.481	0.735
HSI	0.589	35.5	68.1%	53.4%	<0.001*	0.458	0.719
CAP	0.916	0.168	92.1%	82.4%	<0.001*	0.852	0.980
FIB-4	0.811	0.45	76.6%	60.2	<0.001*	0.727	0.896

RBP-4; retinol-binding protein-4, FLI; fatty liver index, HSI; hepatic steatosis index, FIB-4; fibrosis-4 score, CAP; controlled attenuation parameter.*P < 0.05 when compared with the control group. .*P < 0.05

Table S2: Comparison of ROC curve results of different variables to assess their diagnostic power in differentiation of children with NAFLD among obese children

Variables	AUC	Cutoff value	Sensitivity	Specificity	P value	95% C.I	
						lower	upper
Serum RBP4	0.877	58.3	87.1%	81%	<0.001*	0.740	1.000
RBP4 mRNA	0.861	4.8	86.8%	80%	<0.001*	0.724	1.000
FLI	0.726	38.53	84.1%	79.3%	<0.001*	0.497	0.955
HSI	0.857	33.32	85.7%	72.2%	<0.001*	0.740	1.000
CAP	0.889	186.2	86.6%	80.1%	<0.001*	0.761	1.000
PNFS	0.762	15.53	85.5%	72.1%	<0.001*	0.527	0.997

RBP-4; retinol-binding protein-4, FLI; fatty liver index, HSI; hepatic steatosis index, PNFS; Pediatric NAFLD Fibrosis Score, CAP; controlled attenuation parameter.*P < 0.05 when compared with the control group. .*P < 0.05.

Table S3: Comparison of ROC curve results of different variables to assess their diagnostic power in differentiation of adults with NAFLD among obese adults.

Variables	AUC	Cutoff value	Sensitivity	Specificity	P value	95% C. I	
						lower	upper
Serum RBP4	0.595	74.5	66.1%	64.3%	<0.001*	0.310	0.881
RBP4 mRNA	0.585	6.2	65.7%	61.1%	<0.001*	0.310	0.881
FLI	0.500	52.5	655	50%	<0.001*	0.243	0.757
HSI	0.559	50.3	61.1%	57%	<0.001*	0.310	0.881
CAP	0.718	256.4	77.1%	57.8%	<0.001*	0.511	0.926
FIB-4	0.706	0.97	72.2%	82%	<0.001*	0.503	0.909

RBP-4; retinol-binding protein-4, FLI; fatty liver index, HSI; hepatic steatosis index, FIB-4; fibrosis-4 score, CAP; controlled attenuation parameter.*P < 0.05 when compared with the control group. .*P < 0.05

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