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Assessment of interleukin 32 as a novel biomarker for non-alcoholic fatty liver disease

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder characterised by enhanced hepatic fat deposition and inflammation. Efforts to manage NAFLD are limited by the poorly characterised pathological processes and the lack of precise non-invasive markers, thus, proving the need to further study the involved cytokines, which, in turn, may represent novel molecular targets with possible diagnostic and therapeutic applications. Hence, we aimed to assess the diagnostic utility of serum interleukin 32 (IL-32) in NAFLD cases. This case-control study included 40 NAFLD patients and 40 healthy controls. The serum IL-32 concentrations were assessed by the enzyme-linked immunosorbent assay (ELISA).

Results: The serum IL-32 concentrations were significantly higher in NAFLD cases than controls (76 [45.5–111.125] vs. 13 [8–15] pg/mL, $P < 0.001$, respectively). IL-32 at a cut-off point > 22.5 pg/mL had 100% sensitivity, 87.50% specificity, 88.9% positive predictive value, 100% negative predictive value, and 98.2% accuracy in detecting the NAFLD cases.

Conclusion: Serum IL-32 could be considered a novel non-invasive marker for NAFLD. Further investigations are warranted to verify the potential utility of IL-32 in the clinical setting.

Keywords: Non-alcoholic fatty liver disease, NAFLD, IL-32, Marker, Non-invasive, Diagnosis

Background

Non-alcoholic fatty liver disease (NAFLD) is currently a major driver of progressive hepatic disease globally, with a 15–30% prevalence rate. It is considered to be the hepatic component of metabolic syndrome [1, 2]. NAFLD has a broad spectrum of diseases. Simple steatosis is defined as the hepatic accumulation of triglycerides; it accounts for approximately 80% of NAFLD cases and is associated with a good prognosis. Approximately 25% of cases with simple steatosis develop into the more severe stage of non-alcoholic steatohepatitis (NASH), in which liver steatosis coexists with hepatic inflammation that can be accompanied by the ballooning of hepatocytes and liver fibrosis [3, 4]. Eventually, 20% of NASH cases progress to cirrhosis or hepatocellular carcinoma over 20–30 years [5].

The pathogenesis of NAFLD is not yet fully understood. Its existing pathophysiology proposes the multifactorial interaction of various metabolic, genetic and environmental influences, such as the gut microbiota and innate immunity interaction, mitochondrial dysfunction, abnormalities of iron metabolism, and increased fructose consumption [6–9], with the proliferation, dysfunction, and inflammation of adipose tissue [10, 11].

The infiltration of adipose tissue with immune cells results in altered adipokines and promotes metabolic diseases [10, 12]. Moreover, chronic inflammation and insulin resistance stimulate the release of free fatty acids (FFAs) from the adipose tissue, causing hepatocellular fat deposition. Subsequently, inflammatory adipokines promote the deposition of fibrous tissue, which is the hallmark of the disease progressing to cirrhosis [10, 12]. However, the inflammatory response is also essential for tissue repair during the early phases

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of hepatic damage [13]. This dual aspect of the inflammatory system may be an interesting target in the management of NAFLD. For example, interleukins such as IL-6, IL-8, IL-12, IL-18, and IL-34 have been shown to play a part in NAFLD disease [14–18].

Among several inflammatory biomarkers linked to obesity and NAFLD, IL-32 is evolving as a main regulator of obesity-driven inflammation and lipotoxicity. IL-32 was initially discovered in 2005 [19]. Formerly, it was recognised as NK4, a transcript produced after activating natural killer (NK) and T cells by IL-2 and mitogens, respectively [20, 21]. The IL-32 gene exists only in humans on chromosome 16p13.3. It has a full length of approximately 1.2 kbp and comprises eight small exons. IL-32 has nine isoforms which have different and sometimes opposing functions [22, 23].

There is still controversy regarding IL-32 localisation in and secretion from cells [22]. It is found mainly intracellularly [24]. It has been observed that IL-32 is localised in the cytoplasm and nucleus of Leishmania-infected macrophages, the endoplasmic reticulum of endothelial cells [24, 25], the Golgi compartment [26], and in the mitochondria of breast cancer cells [27]. IL-32 has also been found to colocalise with lysosomes [28]. IL-32 has also been observed in the supernatant of activated cells, suggesting that IL-32 is secreted or released from cells [29]. Owing to its localisation in the Golgi compartment, IL-32 may also be secreted across the endoplasmic reticulum-Golgi pathway [26]. However, the secretory path of IL-32 has not been defined in detail nor has a cognate receptor of IL-32 been discovered; the most closely related receptor is the proteinase 3-proteinase activated receptor 2 axis [30]. Consequently, thorough research should be performed to identify the localisation and secretion of IL-32.

IL-32 is expressed in several human tissues, but it is more prominent in immune cells (such as NK cells, macrophages, monocytes, and T cells) than in non-immune cells (such as epithelial cells, endothelial cells, mesenchymal stromal cells, fibroblasts, and hepatocytes) [21, 29, 31]. IL-32 expression is also seen in various cancer cell lines [21, 23, 26, 32]. Although IL-32 can be expressed in normal circumstances, IL-32 expression is robustly stimulated in reaction to the proinflammatory cytokines [28, 33], infections [28, 34], oxidative stress responses [35], and various disorders such as type 2 diabetes [36], allergic rhinitis [37], chronic obstructive pulmonary disease [38], atopic dermatitis [25], systemic lupus erythematosus [39], and rheumatoid arthritis [40]. Regarding hepatic diseases, IL-32 is upregulated in hepatitis C and B viral infections and NAFLD and is also associated with the severity of hepatic disease [41–45].

IL-32 exhibits several biological activities including monocyte differentiation and stimulation of pro/anti-inflammatory cytokines as tumour necrosis factor-α (TNFα) and the activation of nuclear factor kappa (NF-κB) signalling pathways [19, 22]. Conversely, TNFα and interferon γ (IFNγ) have been found to stimulate IL-32 secretion in various pathological conditions [46, 47]. Notably, the mutual positive transcription between IL-32 and TNFα triggered a low-grade inflammation in lipid-storing organs through the enhanced expression of proinflammatory cytokines such as IL-1b, IL-6, and IL-10, whereas IL-32 silencing yielded opposite effects [48]. However, other reports have shown IL-32 to have the opposite function by stimulating anti-inflammatory cytokines such as IL-10 and immunosuppressive molecules such as Indoleamine 2, 3-dioxygenase in immune cells [49].

The function of IL-32 in metabolic conditions is unclear. It may be initially upregulated as a protective response against the accumulation of FFAs. Additionally, the overexpression of IL-32 in HepG2 cells resulted in reduced intracellular fat accumulation, whereas shRNA knockdown of IL-32 enhanced fat accumulation. However, over time, the proinflammatory impacts of IL-32 and its influences on insulin resistance could play an essential part in the pathogenesis of metabolic syndrome [45, 50, 51].

A variety of mechanisms have been proposed to ascertain the role of IL-32 in lipid metabolism. IL-32 promoters have binding locations for fatty acid-responsive transcription factors, which are upregulated during lipotoxicity [44]. IL-32 has been linked to endothelial inflammation in response to the postprandial increase in FFAs [52]. In addition, it has a role in regulating the intracellular content of lipids by modulating cholesterol efflux [53, 54]. Moreover, a positive association between IL-32 mRNA and the lipid regulatory receptor liver X receptor α (LXRα), the lipid transporters ATP-binding cassette subfamily A member 1 (ABCA1), ATP-binding cassette subfamily G member 1 (ABCG1), and the fatty acid carrier ApoA1 in primary liver cells has been detected [31]. Hence, we aimed to estimate the serum IL-32 concentrations in NAFLD cases and assess its diagnostic performance for the detection of NAFLD.

Methods

This case-control study included 40 NAFLD cases and 40 healthy controls, who were recruited from the Internal Medicine and Hepatology outpatient clinics at Ain Shams University Hospitals, Cairo, Egypt, from March 2020 to March 2021. Exclusion criteria were viral hepatitis, autoimmune liver diseases, metabolic liver diseases (hemochromatosis, Wilson's disease, alpha-1-antitrypsin

deficiency, and cystic fibrosis), alcoholic liver disease (quantity of ethanol consumption > 20 g/day for females and > 30 g/day for males), concurrent infections, and concomitant conditions causing secondary steatohepatitis, such as endocrine disorders, primary dyslipidaemia, or malnutrition.

All the participants underwent a detailed medical history, physical examination, laboratory investigations, and ultrasonography [55]. Ultrasonography was performed for the diagnosis of NAFLD [55]. The indices of the fatty liver index (FLI) [56] and NAFLD fibrosis score were calculated as reported previously [57]. According to Bedogni et al., the FLI ranges from 0 to 100; NAFLD is ruled out by an FLI <30 and confirmed with an FLI ≥60 [56]. According to Angulo P et al., NAFLD score < − 1.455 = F0–F2, NAFLD score − 1.455–0.675 = indeterminate score, NAFLD score > 0.675 = F3–F4 [57].

Serum IL-32 was measured by the human IL-32 enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, CA, USA, Cat. No: MBS029816). The detection range = 12.5–400 pg/mL, sensitivity = 2.0 pg/mL, and intra- and inter-assay precision were less than 15%.

The study protocol followed the Declaration of Helsinki ethical guidelines. The study was certified by the Ethical Committee of the Faculty of Medicine, Ain Shams University (FWA 000017585). An informed written approval was gained from all the participants prior to their inclusion in the research.

Statistical analysis

All the results were analysed using SPSS Statistics for Windows, v. 25.0 (Armonk, NY: IBM Corp.). Variables were shown as frequency and percentages for categorical variables, mean and standard deviation for parametric numerical variables, and median (interquartile range) for non-parametric numerical variables. The Student *t*-test, Mann-Whitney test, chi-square test, and Fisher's exact test were used when appropriate. Correlation analyses between serum IL-32 levels and other variables were performed using Spearman's rho. A receiver-operating characteristics (ROC) curve analysis was applied to evaluate the diagnostic accuracy of IL-32 for the differentiation of NAFLD patients. A two-tailed $P < 0.05$ was considered significant.

Results

This study included 40 NAFLD cases with a mean age of 49.40 ± 12.01 years, 67.50% of whom were females, and 40 healthy controls with a mean age of 45.66 ± 10.33 years, 47.50% of whom were females (Table 1).

Serum IL-32 concentrations were significantly higher in the NAFLD cases than controls (76 [45.5–111.125] vs. 13 [8–15] pg/mL, $P < 0.001$, respectively; Table 1 and Fig. 1). Serum IL-32 levels did not differ between NAFLD patients with regard to gender, smoking, co-existing hypertension, FLI scores, or NAFLD fibrosis scores ($P \geq 0.05$; Table 2). Serum IL-32 levels correlated only with albumin levels ($P = 0.029$; Table 3). Fatty liver index and NAFLD fibrosis score of all participants are shown in Tables 4 and 5. IL-32 at a cut-off point > 22.5 pg/mL had 100% sensitivity, 87.50% specificity, 88.9% positive predictive value (PPV), 100% negative predictive value (NPV), and 98.2% accuracy for the differentiation of NAFLD cases (Table 6, Fig. 2).

There were statistically significant differences between the NAFLD cases and controls with regard to co-existing hypertension, weight, body mass index, waist circumference, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, triglycerides, cholesterol, high-density lipoprotein, low-density lipoprotein, FLI scores, and NAFLD fibrosis scores ($P < 0.001$; Table 1).

Discussion

At present, liver biopsy remains the favoured method for evaluating the degree of hepatic necro-inflammation and fibrosis in NAFLD cases. However, this invasive procedure has multiple drawbacks including the high cost, the limited representation of the total liver mass, intra- and interobserver inconsistency, and post-procedural complications [58]. These drawbacks prevent the liver biopsy from being a tool for successive disease evaluation in clinical practice. Alternatively, numerous non-invasive laboratory and imaging methods have been proposed as more appropriate for first-line investigations [59–61]. However, the present non-invasive methods lack sufficient sensitivity and specificity. Therefore, innovative strategies are being considered for biomarker discovery and therapeutic target identification, focussing not only on the extent of hepatic fibrosis but also on hepatic inflammation, which represents one other critical part of hepatic disease pathology [62, 63].

According to the current literature, there is an association between IL-32 and obesity-linked inflammation [44, 46]. Furthermore, IL-32 expression increased in visceral adipocytes subjected to inflammatory stimuli as hypoxia, lipopolysaccharide, and TNF-α. In addition, IL-32 triggered the expression of inflammatory biomarkers in these cells [46]. Serum IL-32 concentrations were also increased in type 2 diabetes and correlated with body mass and fasting blood glucose [36] and decreased after bariatric surgery [48]. Therefore, further investigations are essential to determine whether IL-32 is a possible biomarker for NAFLD.

In the current study, we observed significantly higher serum IL-32 concentrations in the NAFLD cases than in the controls. Moreover, serum IL-32 had a reliable

Table 1 Demographic and clinical characteristics of all participants

	NAFLD cases	Controls	*P* value
Age (years)	49.40 ± 12.01	45.66 ± 10.33	0.140
Sex			
Male	13 (32.50%)	21 (52.50%)	0.070
Female	27 (67.50%)	19 (47.50%)	
Special habits			
Non-smoker	30 (75.00%)	32 (80.00%)	0.592
Smoker	10 (25.00%)	8 (20.00%)	
Hypertension			
No	25 (62.5%)	40 (100%)	< 0.001
Yes	15 (37.5%)	0 (0.00%)	
Weight (kg)	93.96 ± 12.64	72.02 ± 7.37	< 0.001
Height (cm)	165.16 ± 4.69	167.50 ± 6.67	0.074
Body mass index	34.31 ± 4.03	25.70 ± 2.53	< 0.001
Waist (cm)	98.10 ± 10.76	78.12 ± 4.65	< 0.001
Interleukin 32 serum levels (pg/mL)	76 (45.5-111.125)	13 (8-15)	< 0.001
Aspartate aminotransferase IU/L	52.05 ± 12.44	28.72 ± 5.58	< 0.001
Alanine aminotransferase IU/L	60.35 ± 16.92	33.17 ± 4.42	< 0.001
Total bilirubin mg/dL	0.67 ± 0.29	0.72 ± 0.26	0.451
Albumin g/dL	3.55 ± 0.42	4.26 ± 0.45	< 0.001
Total glycerides mg/dL	210.85 ± 23.74	131.80 ± 14.60	< 0.001
Total cholesterol mg/dL	230.32 ± 25.97	170.70 ± 17.53	< 0.001
High-density lipoprotein mg/dL	29.17 ± 3.56	37.15 ± 6.26	< 0.001
Low-density lipoprotein mg/dL	160.65 ± 25.93	106.25 ± 19.03	< 0.001

Variables were shown as frequency and percentages for categorical variables, mean and standard deviation for parametric numerical variables, and median (interquartile range) for non-parametric numerical variables

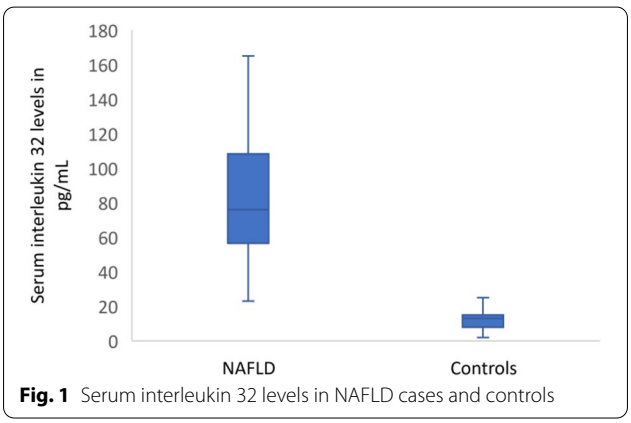

Fig. 1 Serum interleukin 32 levels in NAFLD cases and controls

Table 2 Interleukin 32 levels according to patient characteristics

		Interleukin 32 concentration (pg/mL)		*p* value
		n	Median (IQR)	
Sex	Male	13	76 (49.75–99.5)	0.942
	Female	27	76 (40–120)	
Special habits	Non-smoker	30	76 (39.37–123.75)	0.766
	Smoker	10	76 (65.62–108.37)	
Hypertension	No	25	82 (56.75–13)	0.678
	Yes	15	69 (45.5–191.87)	
Fatty liver index	Indeterminate	7	76 (67.5–200.5)	0.630
	High	33	76 (38.75–105)	
NAFLD fibrosis score	F0–F2	7	76 (76–200.5)	0.318
	Indeterminate	33	76 (38.75–102.25)	

diagnostic accuracy in differentiating NAFLD cases. Similarly, an earlier study showed that serum IL32 concentrations were higher in NAFLD cases compared to controls ($P < 0.01$) [44]. Furthermore, higher serum IL-32 levels were observed in patients with severe NAFLD than those without severe disease ($P < 0.01$) [44]. In the same study, the inclusion of IL-32 in the ALT-AST model caused a 24% increase in AUC for the differentiation of NAFLD (AUC = 0.92 vs. 0.81) [44].

In accordance with the current findings, Dali-Youcef et al. reported that IL-32 protein levels were increased in hepatic samples of NAFLD cases. Additionally, compared

Table 3 Correlations between interleukin 32 and other laboratory data

	Serum interleukin 32	
	r	***p* value**
Age	− 0.170	0.294
Weight	0.087	0.592
Height	0.021	0.900
Body mass index	0.058	0.722
Waist	0.131	0.421
Aspartate aminotransferase	0.293	0.067
Alanine aminotransferase	0.058	0.724
Total bilirubin	0.001	0.997
Albumin	− 0.346	0.029
Total glycerides	− 0.129	0.427
Total cholesterol	0.149	0.358
High-density lipoprotein	− 0.149	0.358
Low-density lipoprotein	0.172	0.289
Fatty liver index	− 0.004	0.982
NAFLD fibrosis score	− 0.026	0.875

Table 4 Fibrosis liver index of all participants

		NAFLD cases	Controls	*p* value
Fatty liver index	Mean ± SD	76.80 ± 17.93	22.17 ± 6.42	< 0.001
	Low *n* (%)	0 (0.00%)	39 (97.50%)	< 0.001
	Indeterminate *n* (%)	7 (17.50%)	1 (2.50%)	
	High *n* (%)	33 (82.50%)	0 (0.00%)	

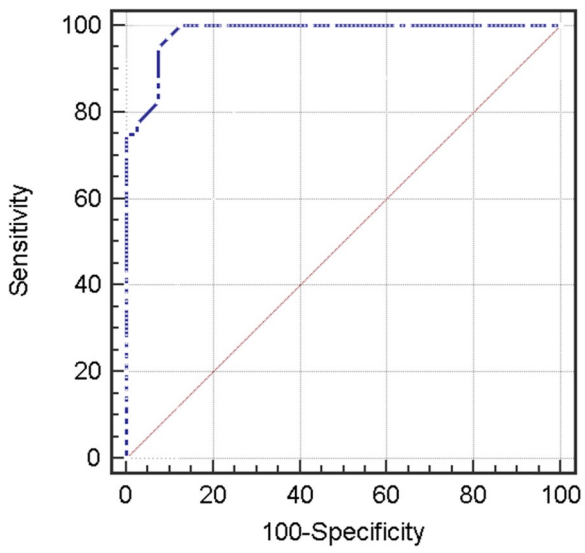

Fig. 2 ROC curve analysis for the diagnostic performance of IL-32 for NAFLD detection

Table 5 NAFLD score of all participants

		NAFLD cases	Controls	*p* value
NAFLD fibrosis score	Mean ± SD	− 0.785 ± 0.80	− 3.21 ± 0.54	< 0.001
	F0-F2 *n* (%)	7 (17.50%)	40 (100%)	< 0.001
	Indeterminate *n* (%)	33 (82.50%)	0 (0.00%)	

Table 6 ROC curve analysis for the diagnostic performance of IL-32 for NAFLD detection

	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum interleukin 32	> 22.5 pg/mL	100%	87.50%	88.9%	100%	98.2%

NPV negative predictive value, *PPV* positive predictive value

to controls, IL-32 expression was raised 2.5-fold in the NAFLD cases ($P < 0.001$), while a statistically non-significant increase in IL-32 expression was detected in obese patients with normal livers (1.6-fold). Dali-Youcef et al. also noticed a positive association between IL-32 and waist circumference, body mass index, aminotransferases, and NAFLD score, suggesting that this gene contributes to liver steatosis and metabolic syndrome [45].

The current study is limited by the small sample size and the lack of a paired histological evaluation of NAFLD due to the invasiveness of liver biopsy. Finally, IL-32 has emerged as an adipokine with a substantial influence on a range of human diseases including NAFLD; this influence suggests IL-32 and its signalling pathway could be a potential therapeutic target [45]. Further large-scale studies should be performed to determine the molecular function of IL-32 and recognition of IL-32 interacting molecules will help achieve this goal [64].

Conclusions

Serum IL-32 could be considered a novel non-invasive marker for NAFLD. Further investigations are warranted to verify the potential utility of IL-32 in the clinical setting.

Abbreviations

ABCA1: ATP-binding cassette subfamily A member 1; ABCG1: ATP-binding cassette subfamily G member 1; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AUC: Area under the curve; ELISA: Enzyme-linked immunosorbent assay; FFAs: Free fatty acids; IFNγ: Interferon γ; IL: Interleukin; LXRα: Lipid regulatory receptor liver X receptor α; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; NK cells: Natural killer cells; NPP: Negative predictive value; NF-κB: Nuclear factor kappa B; PPV: Positive

predictive value; ROC: Receiver-operating characteristics curve; TNF-α: Tumour necrosis factor-α.

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Authors' contributions
MM, SG, GM designed the research; KA participated in the acquisition of data; MM, SG, KA, GM participated in the analysis and interpretation of the data; MM, SG, KA, GM revised the article critically for important intellectual content; GM wrote the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Approval was obtained from the Ethics Committee of the Faculty of Medicine, Ain Shams University (FWA 000017585). Informed written consent was obtained from each participant before enrollment in the study. This study was performed in accordance with the 1975 principles of the Declaration of Helsinki and its appendices.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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