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The predictive role of parathyroid hormone for non-alcoholic fatty liver disease based on invasive and noninvasive findings in candidates of bariatric surgery

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD)**,** non-alcoholic steatohepatitis (NASH) and hepatic fibrosis are the most detrimental hepatic abnormalities associated with increased body weight with devastating clinical outcomes. Therefore, there is a substantial necessity for efficient management strategies, including significant weight reduction. Bariatric surgery has been used as a therapeutic approach in a selected obese patient with NAFLD/NASH and other cardiometabolic comorbidities.

Purpose: The study is focused on the predictive role of PTH with the indices of hepatic steatosis/ NAFLD and NASH based on liver biopsy, elastography and sonography in morbidly obese patients.

Methods: Ninety patients with BMI between 35 and 40 kg/m2 with more than two comorbidities who referred to Imam Reza outpatient clinic from December 2016 to September 2017 were recruited and underwent initial assessments, including demographic profiles, psychological assessment, anthropometric measurements, hepatic biopsy, and basic laboratory tests. Liver stiffness was evaluated using two-dimensional shear wave elastography (2D-SWE) at least 2-weeks before liver biopsy. The histological analysis of the liver was performed using biopsy samples which obtained from left hepatic lobe during bariatric surgery under direct surgeon observation using a 16-gauge Tru-cut needle. The study was approved by the ethical committee (IR.MUMS.fm.REC.1396.312).

Results: The level of PTH was significantly high in patients with positive histology for hepatic fibrosis, steatosis and NASH/NAFLD compared to patients with negative histology (p=0.005,

p=0.009 and p=0.013, respectively). Also, patients with liver fibrosis confirmed by elastography had significantly higher serum PTH concentration than patients without fibrosis (p=0.011). PTH was also positively correlated with hepatic fibrosis, NASH, and steatosis (p=0.007, p=0.012, p=0.023. respectively).

Conclusion: High levels of PTH was significantly associated with histological indices of (hepatic fibrosis, steatosis, NAFLD and NASH) and elasticity indices. Therefore, it is imperative to assess for high levels of PTH in the morbidly obese population pre-and post-bariatric surgery. However, for a more robust and comprehensive assessment, a randomised controlled trial is needed.

The study was conducted in accordance with the practice guidance in the diagnosis and management of NAFLD from the American association for the study of liver disease (AASLD) 2018 .

Level III: Evidence obtained from well-designed cohort or case-control analytic studies.

Keywords: Hepatic fibrosis, Steatosis, NAFLD, NASH, Two-dimensional shear wave elastography, Biopsy, Bariatric surgery.

Introduction

Morbid obesity (BMI> 40 kg/m²) is a primary global health concern associated with increased risk of several comorbidities such as type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and other metabolic disorders. Morbid obesity also associated with the growing prevalence of non-alcoholic fatty liver disease (NAFLD) and its advance consequences from simple steatosis to hepatic fibrosis, hepatic cirrhosis, non-alcoholic steatohepatitis (NASH) and hepatocellular cancer [1]. NAFLD is the most frequent cause of liver disorder with a worldwide prevalence of approximately 11 % range between 10-30 % in the western hemisphere [2, 3]. The prevalence increases significantly in overweight and obese patients undergoing bariatric surgery (50-90 %) [3] .

The current diagnostic approach for chronic hepatic disorders consists of non-invasive modalities, including laboratory profiles including liver enzymes, imaging modalities such as ultrasound elastography and magnetic resonance imaging (MRI), related biomarkers and fibroscan [4, 5]. On the other hand, liver biopsy is the gold standard diagnostic approach which will reveal evidence of perilobular inflammation, hepatocytes ballooning, and Mallory hyaline [6, 7]. Recently, a real-time shear wave elastography (SWE) has been emerged as an imaging procedure to demonstrate the hepatic fibrosis [8]. This novel technique quantifies the stiffness of the hepatic tissue in real-time with the aid of B-mode [9, 10].

The primary goal of managing NAFLD is to improve the hepatic steatosis and to prevent fibrosis [11]. There is no standard treatment strategy; however, controlling risk factors associated with NAFLD, such as insulin resistance (IR) and obesity remain the primary target [12]. Currently, lifestyle modifications, medical therapies and bariatric surgery have been used to manage the risk factors related to NAFLD [13]. Bariatric surgery is indicated in patients with BMI > 40 kg/m² or 35 kg/m² with obesity-related comorbidities [14]. It is the most effective treatment for morbidly obese patients resulting in significant weight loss and improved the liver functions, liver histology, steatosis, and fibrosis [15, 16]. However, bariatric surgery has different effects on body weight and calcium absorption. It significantly reduces body weight but also affects the absorption of micronutrients, including calcium [17, 18]. There is emerging evidence showed that secondary hyperparathyroidism is a common consequence of bariatric surgery [19]. It also known that abnormalities in calcium absorption, vitamin D metabolism and high level of PTH are prevalent after bariatric surgery. Research has also indicated that even prior to bariatric surgery, obese patients have lower calcium level and higher levels of PTH. However, robust data are lacking. Therefore, this study aims to explore the predictive role of PTH in patients with chronic liver disease who underwent bariatric surgery.

The study was conducted in accordance with the practice guidance in the diagnosis and management of NAFLD from the American association for the study of liver disease (AASLD) 2018 [20].

Material and Methods

The same as our previous work, those patients with BMI more than 40 kg/m2 or over 35 with more than two comorbidities who referred to Imam Reza outpatient clinic from December 2016 to September 2017 were recruited in this study. Medical evaluation and psychological assessments were carried out in subjects who completed the informed consent. Inclusion criteria include alcohol consumption less than 30 g/day and 20 g/day in males and females, respectively, no drug-induced liver injury, negative for HBs ag and HCV antibody. Finally, 90 patients were included.

The performed procedures in this study were in accordance with the institutional ethical standards (ethics approval code: IR.MUMS.fm.REC.1396.312) and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. An informed consent was taken from all participants prior to the operation.

Two-Dimensional Shear Wave Elastography

Liver stiffness was measured using 2D-SWE two weeks before liver histology. As instructed by the manufacturer, the procedure was performed by Aixplorer ultrasound system (Supersonic Imagine, France) using a curved broadband probe (SC6-1, 1–6 MHz). For more relevant results, patients were asked to fast six hours before the procedure. They were positioned in right lateral decubitus with a complete abducted right arm to get access liver from intercostal spaces. Ten image acquisitions of each patient were considered as a favourable liver stiffness measurement (LSM). A single operator blinded to the patients' data reported LSM (liver stiffness measurement) as the mean (M) of valid measurements in kilopascals (kPa).

Histologic Analysis of the Liver

Biopsy samples were obtained from left hepatic lobe during bariatric surgery under direct surgeon observation using a 16-gauge Tru-cut needle. Elevated liver function tests, fatty liver confirmed by ultrasound or macroscopic unusual liver tissue were considered as an indication for biopsy. Haematoxylin- eosin-saffron, picrosirius red, Masson's trichrome stains were used to assess biopsy specimens embedded in paraffin by a single expert pathologist blinded to the patient's data. NASH Clinical Research Network Modified Brunt methodology and NASH Activity Score (NAS) were applied for staging and grading of NASH, respectively[21]. Hepatic fibrosis was scored from 0 to 4 based on fibrosis severity, hepatic steatosis was graded from 0 to 3 relying on percentage of liver steatosis (0, < 5%; 1, 5–33%;

2, 34–66%; 3, > 66%), number of diagnosed foci in a × 20 magnitude in lobular inflammation (Scored from 0 to 3; 0: none,1:1–2, 2: 2–4,3: > 4) and number of ballooned hepatocytes in hepatocellular ballooning (scored from 0 to 2; 0: none, 1: few, 2: many). NAS score was ascertained for each subject by a combination of scores as mentioned above. Subsequently, patients were categorised in three groups as follows: no NASH (0-2 points), and definite NASH (3-8)[21, 22].

Statistical Analysis

Descriptive statistics were used to describe demographic variables. Mean (standard deviation [SD]) and median (interquartile range [IQR]) were applied for parametric and non-parametric values, respectively. The association between ordinal data was represented using spearman's coefficient. To demonstrate the diagnostic accuracy of PTH (parathyroid hormone), and define the optimal cut off point, receiver operating characteristic (ROC) curves were plotted. Sensitivity, specificity, and areas under the ROC curves (AUROCs) for the corresponding data were also determined through DeLong's method for correlated data. SPSS (version 25) was used for statistical analysis. Subsequently, the predicted PTH cut-offs were constructed, and AUC was calculated. The p-value for all tests, if applicable, was considered significant at the level of 5%.

Results

Patient Characteristics

Among the 90 patients, the mean age was 38.5 ± 11.1 years, and the mean BMI was measured at 45.46 ± 6.26 kg/m2. More than half (51.9%) had metabolic syndrome; 38 patients had no fibrosis (F < 1) and 52 had a spectrum of liver fibrosis (F ≥ 1). Severe steatosis (> 66%) was observed in 8.9%, and NASH was found in more than half **(Table 1)**.

PTH concentration based on fatty liver disease.

The comparison of serum PTH concentration between study groups is presented in **Table 2**.

The Mean±SD level of serum PTH in patients with positive histology for liver fibrosis, steatosis and NASH were significantly more than patients with negative histology (p=0.005, p=0.009 and p=0.013, respectively). Also, patients with liver fibrosis confirmed by elastography had substantially higher serum PTH concentration than patients without fibrosis (p=0.011).

The Relationship Between PTH and liver status

The relationship between PTH and liver fibrosis (biopsy), NASH (biopsy), steatosis (biopsy), fibrosis (elastography), and steatosis (ultrasonography) are presented in **Table 3**. PTH levels were positively correlated with liver fibrosis (biopsy), NASH (biopsy), steatosis (biopsy) (p=0.007, p=0.012, p=0.023. respectively). Also, liver fibrosis, confirmed by elastography, was correlated with serum PTH concentration (p=0.011, CC: 0.241).

Diagnostic yield of hs-CRP in assessing the liver disease.

The values were determined using the ROC curves as optimal cut-off points. The sensitivity, specificity for each NASH CRN-modified BRUNT methodology stage are shown in **Table 4.** The ROC curve was used to identify the sensitivity and specificity of PTH for liver fibrosis (biopsy) (p=0.001), NASH score (p=0.004), liver steatosis (biopsy) (p=0.003), liver fibrosis (elastography) (p=0.007), and liver steatosis (ultrasonography) (p=0.246).(**Table 4 and Figure 1)**. Based on the ROC curve, the optimal cut-off in the PTH level for detecting fibrosis (biopsy), NASH, Steatosis (biopsy), Fibrosis (elastography), Steatosis (ultrasonography) were 20.8, 20.8, 20.8, 59 and 41.51, respectively.

The binary logistic regression analysis between hs-CRP and study parameters

Binary logistic regression analysis for PTH studied after adjusting by age, sex, WC, AST, ALT, GGT, ALP, and HOMA-IR on subject groups **(Table 5)**. Binary logistic regression analysis showed that serum PTH level was a predictive factor for liver histology in both adjusted and unadjusted models (unadjusted model; p=0.008, p= 0.017 and p=0.012 for NASH, fibrosis, and steatosis, respectively). (adjusted model; p=0.015, p= 0.007 and p=0.015 for NASH, fibrosis, and steatosis, respectively). Furthermore, the serum PTH level was a predictive factor for liver elastography in both models (p=0.014 and p= 0.017 for crude and adjusted models, respectively.

Discussion

In morbidly obese patients, the prevalence of NAFLD is over 90% higher than the healthy populations [23]. Evidence showed that a lower level of vitamin D and high PTH are both correlated negatively with the free fat mass and fat distribution [24]. A case-control study of 100 Egyptians patients with NAFLD reported a significantly low level of vitamin D and increased PTH compared to control [25]. In morbidly obese patients, the elevated level of PTH was a predictive factor for NASH, particularly in those seeking surgical treatment for obesity [26]. The level of increased PTH was dramatically high in patients with positive histological and imaging evidence of liver fibrosis, steatosis, and NASH. Also, obese individuals have a more elevated serum PTH level, probably because of PTH resistance in this population [27]. Bariatric surgery has been a practical option for managing the comorbidities associated with obesity. The incidence of high PTH was 50% and 70% in patients who underwent Roux-en-Y Gastric Bypass (RYGB) and Single Anastomise Gastric Bypass (SAGB), respectively [19, 28]. These findings are supporting the need for bone health assessment in patients awaiting bariatric surgery, particularly in patients with NAFLD.

The high levels of PTH may be due to the high incidence of vitamin D deficiency as a result of NAFLD or due to the impairment of calcium absorption after bariatric surgery. Typically, calcium absorption mostly occurs in the duodenum section of the small intestine with the aid of vitamin D, which enhances its absorption [29]. The liver plays an essential part in vitamin D metabolism. Therefore, chronic liver conditions affect the hepatic regulation of vitamin D metabolism [30]. On the other hand, bariatric surgery, especially gastric bypass surgery, prevent food from getting into this portion of the intestine which drives to calcium-malabsorption. In severely obese patients, the PTH is high independent of bariatric surgery or NAFLD[31]. In a study of obese women with PCOS showed an increased level of PTH and reduced vitamin D compared to women without PCOS [32]. Another study of morbidly obese patients with metabolic syndrome and insulin resistance reported a high level of PTH independent of vitamin D level [33]. On the other hand, high PTH level but not vitamin D was an independent predictor of metabolic syndrome in morbidly obese patients [34].

An increased level of PTH leads to increased production of vitamin D, which enhances calcium absorption and increase bone resorption [35]. Considering these findings, we find

that high levels of PTH in patients with NAFLD before bariatric surgery are mostly attributed to impaired calcium absorption and the lack of vitamin D metabolism due to the hepatic abnormalities.

In this study, we reported that the level of PTH was significantly higher in patients with positive histology of liver steatosis, fibrosis, and NASH compared to patients with negative histology. Moreover, patients with hepatic fibrosis confirmed by elastography had significantly higher PTH levels than patients without fibrosis. Even more, there was a strong correlation between the serum levels of PTH and the status of the liver. High levels of PTH positively correlated with the histologic and sonographic evidence of hepatic fibrosis, steatosis, and NASH. These findings were further supported by a study in which reported vitamin D inadequacy and secondary hyperparathyroidism (high PTH) in patients with chronic liver disease [36].

Furthermore, the prevalence of high PTH in morbidly obese patients before bariatric surgery was primarily due to vitamin D deficiency, and this prevalence was also continued to increase after bariatric surgery especially in patients underwent SAGB and RYGB [16]. The American Society for the metabolic and bariatric surgery guidelines recommended that vitamin D and calcium supplements are necessary for bariatric patients [37]. This highlighted the importance of the long-term follow-up of the patients with bariatric surgery. Even though our study presented the phenomenon of high PTH levels before bariatric surgery in patients with NAFLD, the actual clinical significance of high PTH levels is still not clear. Moreover, it is still not clear whether bariatric surgery and chronic liver disease cause osteoporosis and increase the risk of fragility fractures remained elusive. However, a previous study reported no statistically significant increase in the risk of bone fracture after bariatric surgery [38]. Nevertheless, another study found that bariatric surgery increases the risk of bone fracture, particularly with bypass techniques [39]. Therefore, the clinical importance of high levels of PTH pre-and-post bariatric surgery in patients with NAFLD remained undetermined, and it requires rigorous and further studies.

Our study has some limitations. Our limitation is that we are unable to provide a thorough analysis of the effect of food intake, vitamin D and calcium supplement on the level of PTH.

More data regarding bone fracture and osteoporosis are required. The strength of the study is that we used clinically robust technique, including biopsy and elastography, to determine the hepatic pathology in a reasonably large number of cohorts.

There are a significant amount of evidence strongly support the notion of elevated serum PTH as a predictive factor for NASH in obese patients seeking bariatric surgery. However, there is lack of evidence supporting the needs for pre-operative assessment for high level of PTH in morbidly obese patients seeking bariatric surgery. Therefore, this study is affirms the significance and the need for evaluating the level of PTH in every candidate for bariatric surgery with great implication in clinical practice.

Conclusion

In summary, the study found higher levels of serum PTH in patients with positive histology for liver fibrosis, steatosis, and NASH than patients with negative histology ($p=0.005$, $p=0.009$ and $p=0.013$, respectively). There is also a strong association between high levels of PTH and liver fibrosis (biopsy), NASH (biopsy), and steatosis (biopsy) ($p=0.007$, $p=0.012$, $p=0.023$. respectively) in morbidly obese patients candidates for bariatric surgery. Therefore, the finding of this study add to the notion that obese patients have higher PTH levels. It also support the significance of the pre-operative evaluation and the need for assessment for secondary hyperparathyroidism and osteoporosis in patients with hepatic fibrosis, steatosis, and NASH before and after bariatric surgery. The results will also have a significant implication in both clinical practice and public health as obesity became more prevalent and so does NAFLD.

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Conflict of interest

None of the authors reports any conflict of interest regarding this manuscript.

Ethics approval

Ethical approval was obtained.

Consent to participate.

Appropriate consent was obtained before participating in the study.

Consent for Publication

All authors are consenting for the publication of the manuscript.

Data availability

All data related to this study are available upon request from the correspondent author.

Authorship

T.J. conceptualisation & methodology, M.N. conceptualisation & methodology A.J. conceptualisation & methodology, L.G. conceptualisation & methodology ,M.A. writing-review and editing, S. A. conceptualisation & methodology ,T. S. supervision; writing-review and editing. ,A. S. conceptualisation & methodology.

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Table 1. Patient Characteristics		
Variable		**Total (n = 90)**
Male		18(20%)
Age		38.5 ± 11.1 years
BMI		45.46 ± 6.26 Kg/m²
Weight		121.34 ± 20.32 Kg
Waist Circumference		133.04 ± 13.6 cm
Height		1.62 ± 8.87 m
Diabetes Type 2		25 (27.8)
Hypertension		23 (25.6)
Metabolic syndrome		46 (51.1)
Liver stiffness measurement (kPa)		6.1 ± 1.25
AST(IU/L)		23.60±10.48
ALT(IU/L)		26.69±19.30
GGT(IU/L)		31.62±20.21
ALP(IU/L)		196.25±53.79
HOMA-IR(mg/l)		6.28±6.77
Fibrosis stage	0 = No fibrosis	38 (42.2)
	1 = Zone 3 perivenular or pericellular fibrosis	40 (44.4)
	2 = Stage 1 plus portal fibrosis	8 (8.8)

	3 = Bridging fibrosis, focal or extensive	4 (4.4)
	4 = Residual pericellular fibrosis	-
NASH status	No NASH (0–2)	39 (43.3)
	NASH (3–8)	51 (56.7)
Steatosis status	S0 = <5%	39 (43.3)
	S1 = 5–33%	31 (34.4)
	S2 = 34–66%	12 (13.3)
	S3 = > 66%	8 (8.9)

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP, alkaline phosphatase; BMI: body mass index; GGT, gama-glutamyl transferase; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; NASH: Non-alcoholic steatohepatitis. Data presented as mean ± SD or number (percent).

Table 2. Mean±SD of serum PTH in liver disease

Variable		Mean±SD	P-value
Fibrosis (biopsy)	Yes	53.26 ± 26.39	0.005
	No	37.07 ± 24.91	
NASH (biopsy)	Yes	52.08 ± 26.08	0.013
	No	37.88 ± 25.05	
Steatosis (biopsy)	Yes	52.98 ± 26.52	0.009
	No	37.88 ± 25.05	
Fibrosis (elastography)	Yes	53.29 ± 28.72	0.011
	No	38.17 ± 22.99	
Steatosis (ultrasonography)	Yes	48.32 ± 27.42	0.311

	No	41.21 ± 26.71	

SD: Standard deviation; PTH: Parathyroid hormone. Data presented as mean±SD.

Table 3. Correlation coefficient between parameters

	PTH	
CC	**r**	**p-value**
Fibrosis (biopsy)	0.287	0.007
NASH (biopsy)	0.270	0.012
Steatosis (biopsy)	0.243	0.023
Fibrosis (elastography)	0.241	0.027
Steatosis (ultrasonography)	0.140	0.208

PTH: Parathyroid hormone; CC: correlation coefficient; NASH: Non-alcoholic steatohepatitis

Pearson correlation was statistically significant at 5 %.

Table 4. Diagnostic performance of PTH vs Liver disease

Diagnostic performance		AUC	cut-off	Sens	Spec
PTH	Fibrosis (biopsy)	0.69	20.8	94.1%	41.7%
	NASH score	0.67	20.8	93.9%	40.5%
	Steatosis (biopsy)	0.68	20.8	94%	40.5%
	Fibrosis (Elastography)	0.66	59	42.6%	83.8%
	Steatosis (ultrasonography)	0.58	41.51	56%	65%

AUC: Area under the curve; Sens: sensitivity; Spec: Specificity; PTH: Parathyroid hormone; NASH: Non-alcoholic steatohepatitis.

Figure 1. The ROC curve for PTH in the detection of liver disease

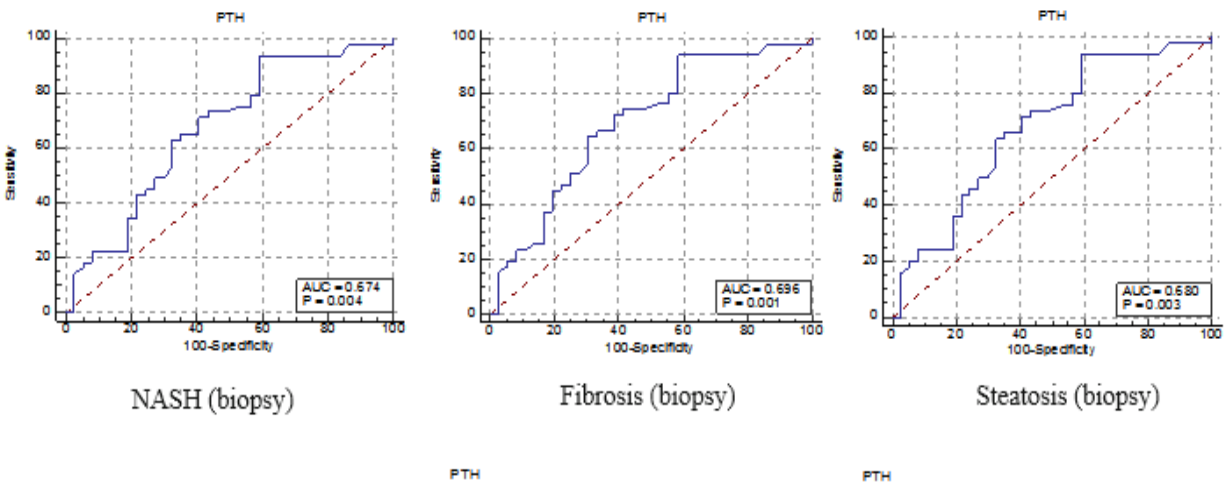

Table 5. The binary logistic regression analysis between PTH and study parameters

Parameters		*p*	OR	95% CI for OR	
				Lower	Upper
Crude Model	NASH	0.017	1.023	1.004	1.042
	Fibrosis (biopsy)	0.008	1.026	1.007	1.046
	Steatosis (biopsy)	0.012	1.024	1.005	1.043
	Fibrosis (Elastography)	0.014	1.023	1.005	1.042
	Steatosis (ultrasonography)	0.309	1.010	0.990	1.031
Adjusted Model	NASH	0.015	1.035	1.007	1.065
	Fibrosis (biopsy)	0.007	1.042	1.011	1.073
	Steatosis (biopsy)	0.015	1.035	1.007	1.065
	Fibrosis (Elastography)	0.017	1.035	1.006	1.064
	Steatosis (ultrasonography)	0.573	1.008	0.980	1.037

*The binary logistic regression analysis between PTH and age, sex, WC, AST, ALT,GGT, ALP, and HOMA-IR on subject groups. P value significant at 0.05.