Research Paper

CLINICAL INVESTIGATION

Liver enzymes in patients diagnosed with non-alcoholic fatty liver disease (NAFLD) in Veracruz: a comparative analysis with the literature

Abstract

Background: Up to the present, NAFLD diagnosis has been established through the invasive method of biopsy. The search for a non-invasive alternative, for example biomarkers, is a motive for research. Previous studies have shown that for diabetic or obese patients with NAFLD, the profile of liver enzymes as NAFLD biomarkers has so far not defined pathological liver condition as such. This study aim was comparatively analyze the results of previous research on liver function enzymes of obese patients with NAFLD and to corroborate them with this type of patient in Veracruz.

Methods and Findings: Forty hospital patients were recruited and classified according to their body mass index (BMI) into 4 groups: Group A (Overweight), Group B (Grade I obesity), Group C (Grade II obesity) and Group D (Grade III obesity), leaving aside those who had a history of hepatitis or consumption of alcoholic beberages. A survey was conducted on these patients to determine sex, signs of liver disease, medicine consumption; laboratory studies which included glucose, total cholesterol, triglycerides, alanine aminotranferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), total protein, and total bilirrubin were also determined. Glucose levels were directly related to BMI, and the most frequent disorder was elevation of ALT levels (72.5%), higher than AST (25%) and ALP (45%). Some variables of the lipid profile showed a significant ($P<0.05$) elevation of triglycerides (85%) and highly significant ($P<0.01$) raised cholesterol levels (82.5%).

Conclusions: The study shows that there are alterations in liver enzymes levels and highlight the importance of gender, BMI and dyslipidemia to assess the risk of individuals with NAFLD, which reaffirm the association with the disease.

Keywords: Hepatic enzymes ▪ Non-alcoholic fatty liver disease ▪ Obesity ▪ Type 2 diabetes

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Introduction

Fatty liver disease is defined as the accumulation of macrovesicular fat that increases liver weight by 5-10%. It encompasses a spectrum of disorders ranging from fat accumulation or steatosis and hepatic inflammation (steatohepatitis), to fibrosis and even cirrhosis, with all the complications that

entail, such as portal hypertension and hepatocellular carcinoma [1,2]

There are two major groups of fatty liver, either alcoholic or non-alcoholic [3-5]. The latter, known as non-alcoholic fatty liver disease (NAFLD), describes the accumulation of lipids in hepatocytes of subjects who drink little or no alcohol. It is mainly associated with metabolic diseases such as type 2 diabetes mellitus, obesity, dyslipidemia and other components of the metabolic syndrome [6]. Alcohol consumption deemed as the definition of non-association with alcohol in men is less than 40 mL per day and less than 20 mL per day for women [4,7]. NAFLD prevalence has been increasing in recent decades. Although the NAFLD condition in Mexico has not been clearly defined, there are reports where it is detected in 10.3% of the general population and 18.5% of the diabetic population [1]. NAFLD is characterized by the persistent rise in hepatic enzymes, without association to excessive consumption of alcohol; it is asymptomatic, with specific ultrasound and histological characteristics, when there is an excess of fat within the hepatocyte [4,7]. The diagnosis is usually made by a Para clinical study, where there is a persistence of elevated liver enzymes. Particular alanine amino transferase (ALT) and aspartate amino transferase (AST) are present and through hepatic ultrasound it is detected an increase of the refringence with respect to the kidney. However, it is the liver biopsy that allows diagnosis confirmation and the study of the degree of severity [7-9].

Classically significant risk groups where a condition of NAFLD could be diagnosed have been described as diabetic, obese or dyslipidemic [10-12]. Today it is known that NAFLD can also occur in people who have no apparent risk factors, but whose fat distribution is altered, as in the case of subjects who have a tendency to predominantly abdominal fat distribution, still being of slim build; so the clinical spectrum is wider than anyone originally thought [7].

Up to the present, NAFLD diagnosis has been established through the invasive method of biopsy. The search for a non-invasive alternative, for example biomarkers, is a motive for research. Previous studies have shown that for diabetic or obese patients with NAFLD, the profile of liver enzymes as NAFLD biomarkers has so far not defined pathological liver condition as such. Therefore, the objective of this study was to comparatively analyze the results of previous research on liver function enzymes in obese patients with NAFLD and to corroborate them with this type of patients in Veracruz ISSSTE General Hospital.

Methods

Selection and processing of biological samples

The frequency of liver enzymes was evaluated using clinical files and the Munich Alcoholism Test (MALT) for patients of the Veracruz ISSSTE General Hospital with a confirmed non-alcoholic fatty liver diagnosis. Essential information concerning to risk factors such as sex, anthropometric measurements and the presence of diabetes were also collected. The group was made up of 21 men and 19 women. They were classified according to body mass index (BMI) into 4 groups: Group A (n=7), overweight patients with fatty liver; Group B (n=8), patients with fatty liver disease and grade I obesity; Group C (n=17), patients with fatty liver disease and grade II obesity, and Group D (n=8), patients with fatty liver disease and grade III obesity.

The eligibility criterion used to define and classify obesity according to the World Health Organization (WHO, 2000) was body mass index (BMI, kg/m2): Normal weight (18.5-24.9); Overweight (25-29.9); Grade 1 Obesity (30–34.9), Grade II Obesity (35-39.9), Grade III Obesity (≥40). Additionally, the MALT test was used to classify the patient as non-alcoholic, a non-inclusion criterion considered to be patients with ≥11 points. Other considerations were not having suffered from hepatitis for at least six months previously, not to be in treatments using contraceptives, paracetamol, aspirin, gold salts, phenylbutazone and non-steroid antihistamines, and, in general, non-use of hepatotoxic drugs.

The participants were informed regarding the study and after clarifying the explanation of the protocol, fasting by all subjects was required before sampling. A sample of 10 ml venous blood was taken from the antecubital vein and placed in tubes. Blood was centrifuged at 35,000 rpm for 15 min to retrieve the serum and immediately the analyte profile was determined. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Veracruz ISSSTE General Hospital.

Assays

Glucose was determined by the glucose oxidase method; total cholesterol (TC), triglyceride (TG), total protein, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined with COBAS c 501 (Roche Diagnostic, USA) through enzymatic colorimetric methods using BioMérieux (Paris, France) kits.

Statistical analysis

Data are expressed as the mean ± standard deviation ($\bar{}$ ± SD). Statistical significance was determined by ANOVA procedures, with post hoc Tukey multiple range tests for comparison of ranges ($P < 0.05$). Data were analyzed with a 2011 IBM© SPSS© 20 version statistical package.

Results

General characteristics of the study population

Table 1 shows the general characteristics and biochemical markers in patients diagnosed with NAFLD. A total of 40 patients were included, 21 men (52.5%) and 19 women (47.5%). Average body mass index (BMI) was 35.4±4.6. Most of the patients were overweight

Table 1. General characteristics and blood markers in patients diagnosed with NAFLD

N	Sex	BMI	BMI Classification	Diabetes	Glucose (mg/mL)	TC (mg/dL)	TG (mg/dL)	ALT (U/L)	AST (U/L)	ALP (U/L)	BT (mg/dL)	PT (mg/dL)
1	M	28	Overweight	NO	89	278	189	46	35	367	0.65	7.9
2	M	29	Overweight	NO	96	204	156	35	25	156	0.99	6.9
3	M	28	Overweight	NO	100	187	145	36	24	158	0.93	7.9
4	F	29	Overweight	Yes	110	190	146	39	38	145	0.92	6.4
5	M	28	Overweight	NO	85	199	123	36	32	90	0.95	7.7
6	M	29	Overweight	NO	92	185	134	25	21	95	0.97	7.5
7	F	24	Overweight	NO	98	178	145	36	32	279	0.78	7.0
8	F	33	Obesity grade 1	Yes	135	189	139	26	20	129	0.83	7.9
9	M	34	Obesity grade 1	Yes	143	178	289	76	68	79	0.89	6.4
10	F	33	Obesity grade 1	Yes	176	299	204	47	40	115	0.78	8.3
11	M	31	Obesity grade 1	NO	94	246	278	76	63	289	1.9	6.8
12	F	32	Obesity grade 1	NO	89	267	278	54	35	325	0.85	8.1
13	F	33	Obesity grade 1	NO	90	256	289	49	31	267	1.4	7.2
14	F	34	Obesity grade 1	NO	91	354	167	48	36	210	0.82	8.1
15	F	34	Obesity grade 1	NO	103	310	199	34	24	156	0.92	7.6
16	F	36	Obesity grade II	NO	98	256	190	43	34	256	1.2	6.9
17	M	39	Obesity grade II	Yes	167	300	256	49	41	245	0.89	6.9
18	F	35	Obesity grade II	NO	85	367	278	59	45	289	0.73	6.0
19	M	37	Obesity grade II	Yes	149	296	256	46	35	167	0.94	7.2
20	M	35	Obesity grade II	NO	100	345	210	80	69	278	1.6	7.4
21	F	37	Obesity grade II	NO	91	345	299	65	53	305	0.79	6.8
22	M	37	Obesity grade II	Yes	118	270	200	37	25	182	1.5	6.9
23	M	36	Obesity grade II	Yes	134	289	256	39	21	164	0.78	6.9
24	M	35	Obesity grade II	NO	98	367	245	36	26	174	0.85	8.6
25	F	38	Obesity grade II	NO	89	327	198	58	47	278	0.93	7.6
26	M	39	Obesity grade II	Yes	110	338	167	47	39	229	0.65	8.2
27	M	39	Obesity grade II	NO	102	311	178	45	32	189	0.72	7.4
28	M	37	Obesity grade II	Yes	189	342	156	47	29	200	1.1	6.5
29	F	36	Obesity grade II	NO	87	365	164	43	27	196	0.69	7.3
30	F	35	Obesity grade II	NO	102	278	162	47	41	289	0.78	7.3
31	F	35	Obesity grade II	Yes	145	270	189	57	43	295	0.89	6.9
32	F	38	Obesity grade II	NO	102	324	256	32	19	132	1.1	7.3
33	M	41	Obesity grade III	Yes	135	200	178	56	39	56	1.1	7.1
34	M	42	Obesity grade III	Yes	135	295	165	59	45	304	1.3	7.5
35	F	41	Obesity grade III	Yes	165	365	345	58	32	289	0.81	7.4
36	M	42	Obesity grade III	Yes	206	210	200	58	38	56	1.1	7.4
37	M	41	Obesity grade III	Yes	135	340	300	56	39	289	0.95	7.1
38	F	42	Obesity grade III	Yes	148	365	310	59	39	304	1.07	7.5
39	M	42	Obesity grade III	Yes	135	300	170	56	38	141	1.1	7.1
40	F	41	Obesity grade III	Yes	145	365	165	59	39	289	1.1	7.5

BMI, body mass index; TG, triglycerides; TC, total cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase;

BT, total bilirubin; PT, total protein.

(7, 17.5%) and some degree of obesity (33, 82.5%) according to the criteria lay down by the World Health Organization (WHO). The presence of diabetes and two elements of dyslipidemia was found in 19 cases (47.5%), 34 (85%) with hypertriglyceridemia and 33 (82.5%) with hypercholesterolemia respectively. Average levels of ALT, AST, and ALP were (in U/L) 48.8±12.9, 36.4±11.8, and 211.4±82.9, respectively. BT and PT levels were within normal ranges (1.0±0.3 and 7.3±0.6 mg/dL respectively).

Distribution of the patients in the study of the Veracruz ISSSTE General Hospital by BMI

Table 2 shows the distribution of patients with NAFLD classified by BMI. 17.5 percent are overweight (Group A), followed by 20% presenting grade I obesity (Group B), 42.5% with grade II obesity (Group C) and 20% with grade III obesity (Group D).

Biochemical parameters in patients with non-alcoholic fatty liver

Table 3 shows the results found for glycemic levels, lipid profile and liver function enzyme profile in patients with non-alcoholic fatty liver.

Overweight patients with non-alcoholic fatty liver (Group A)

This group was made up of a total of 7 patients, 5 male and 2 female, all overweight and a BMI average of 28±1.8 Kg/m^2. Within this group, two patients presented alterations outside normal ranges in the concentrations of glucose, triglycerides and cholesterol (110, 173, 241 mg/dL, respectively) and in the levels of ALT (46 U/L) and alkaline phosphatase (367 and 279 U/L) for one and two patients respectively. However, the average levels of triglycerides were significantly lower (P≤0.05) in comparison with groups B and D. Additionally, cholesterol levels were significantly lower (P≤0.05) in relation to group B, and very significantly lower (P≤0.01) in comparison to groups C and D.

Patients with non-alcoholic fatty liver disease and grade I obesity (Group B)

This group was made up of 8 patients, 2 male, and 6 female, all with grade I obesity and a BMI average of 33±1.1 Kg/m^2. In this group, 3 patients were diagnosed with diabetes, with glucose levels between the ranges of 135-176 mg/dL, an alteration in ALT enzymes (47-76 U/L) in 6 patients, in AST levels (63 and 68 U/L) in 2 patients, for alkaline phosphatase (267-325 U/L) in 3 patients; and for total bilirubin (1.4, 1.9 U/L) in 2 patients. However, when comparing the average levels among study groups, no significant difference was found for any of these parameters or total proteins. On

Table 2. Distribution of patients with non-alcoholic fatty liver disease grouped according to body mass index.

	Body Mass Index									
Gender	Group A Overweight		Group B Grade I Obesity		Group C Grade II Obesity		Group D Grade III Obesity			
	n	%	n	%	n	%	n	%	n	%
Male	5	12.5	2	5.0	9	22.5	5	12.5	21	52.5
Female	2	5.0	6	15.0	8	20.0	3	7.5	19	47.5
Total	7	17.5	8	20.0	17	42.5	8	20.0	40	100

Table 3. Glucose, lipid profile, and liver enzymes in patients with non-alcoholic fatty liver disease.

	Non-alcoholic fatty liver groups			
Parameter	Group A	Group B	Group C	Group D
Glucose (mg/dL)	96 ± 80	115 ± 32	116 ± 30	150 ± 25
Triglycerides (mg/dL)	148 ± 21	230 ± 60*	215 ± 45	229 ± 76*
Total cholesterol (mg/dL)	203 ± 34	262 ± 60*	317 ± 36**	305 ± 68**
Total protein (mg/dL)	7.3 ± 0.6	7.6 ± 0.7	7.2 ± 0.6	7.3 ± 0.2
Total bilirubin (mg/dL)	0.9 ± 0.1	1 ± 0.4	0.9 ± 0.3	1.1 ± 0.1
Aspartate aminotransferase (U/L)	30 ± 6.3	39.6 ± 17.3	36.8 ± 12.7	38.6 ± 3.5
Alanine aminotransferase (U/L)	36.1 ± 6.2	51.3 ± 17.7	48.8 ± 11.9	57.6 ± 1.4
Alkaline phosphatase (U/L)	184.3 ± 102	196.3 ± 90.1	227.5 ± 55.5	216 ± 112

Values are mean ± SD.

Group A (n=7), overweight patients with fatty liver; Group B (n=8), patients with fatty liver disease and grade I obesity; Group C (n=17), patients with fatty liver disease and grade II obesity, and Group D (n=3), patients with fatty liver disease and grade III obesity. *P<0.05; **P<0.01 compared to Control Group A.

the other hand, almost all patients except one presented hypertriglyceridemia (167-289 mg/dL), whose average of triglycerides (230 ± 60 mg/dL) increased significantly (P<0.05) in relation to groups A and C but not for group D. In addition 6 patients presented hypercholesterolemia (246-354 mg/dL), whose average levels of cholesterol (262±60 mg/dL) increased significantly (P<0.05) in relation to group A. However, when compared to groups C and D (P<0.01 difference compared to group A), these group B cholesterol levels were lower in significance, P<0.05.

Patients with non-alcoholic fatty liver disease and grade II obesity (Group C)

This group was made up of 17 patients, 9 male, and 8 female, all with grade II obesity and an average BMI of 37±1.5 Kg/m^2. Seven patients were diagnosed with diabetes, the levels of which were in the range of 110-189 mg/dL. In this group, all patients presented dyslipidemia, whose average triglyceride and cholesterol levels were raised in comparison to group A. However, the triglycerides in this group were significantly lower (P<0.05) than those of groups B and D, but not of Group A. Contrary to this, cholesterol levels increased significantly (P<0.01) compared to group A, but not to groups B and D. Liver enzyme levels were altered: ALT, 43-80 U/L for 13 patients, AST, 41-69 U/L for 7 patients, alkaline phosphatase, 245-305 U/L for 8 patients and total bilirubin 1.2-1.6 mg/dL for 4 patients. However, no significant differences between the average values of these parameters and levels of total protein were found when study groups were compared.

Patients with non-alcoholic fatty liver disease and grade III obesity (Group D)

This group was made up of 8 patients, 5 males and 3 female, all with grade III obesity and an average BMI of 41±0.5 Kg/m^2. In this group, patients presented alterations in almost all levels of the evaluated parameters: glucose (135-206 mg/dL), triglycerides and cholesterol (165-345 and 200-365 mg/dL), ALT (56-59 U/L) for all patients respectively, and alkaline phosphatase (289-304 U/L for five patients). However, the average triglyceride (229±76 mg/dL) and cholesterol levels (305±68 mg/dL) were higher in comparison, the former significantly (P<0.05) compared to groups A and C, but not for Group B, and the latter very significantly (P<0.01) in comparison to group A, of higher significance when compared to group B (P<0.01 rather than P<0.05) and of equally very high significance in the case of group C.

Discussion

NAFLD is a problem of global health that affects 10 to 24% of the general adult population in several countries [12,13]. However, prevalence increases from 57.5% in obese adults [14] to 75% [15]. Prevalence is 2.6% in children, increasing from 22.5 to 52.8% in obese children [16,17]. Increases between 55 and 70% are also found in people with BMI above 30 Kg/m^2.

When anthropometric variables were considered, the results of this study reveal that 82.5% of the subjects evaluated showed one of the grades of obesity and 17.5 % were overweight. Studies such as Martinez et al. [18] reported obesity in a range of 40 to 100%; however, Rousch et al. [1] in a similar study in Veracruz, found values of 14 t.6%, extremely low compared to the results found in this study. Contrary to this, Uscategui et al. [19] in his study of patients with NAFLD, reported that 73 percent were obese, noting a higher prevalence of obesity in males. Other studies have shown that the prevalence of NAFLD in obese patients is 4.6 times higher, up to 74% [20]. In this sense, the group of patients with NAFLD in this study showed a four-fold prevalence, up to 82.5%, similar to the findings of Bellentani et al. [20] Also, it has been shown that NAFLD increases proportionally with BMI; in this study, eight patients with NAFLD were found to have a BMI >40 Kg/m^2.

The number of males was similar to females, 52.5%, and 47.5% respectively, although Gaviria et al. [21] reported a frequency of 73% for females and 27% for males. The results suggest sex is not a risk factor for predisposition to NAFLD. However, population studies in the USA have shown that the prevalence is higher in men than in women [3], similar to what was found in this study, which is in turn consistent with that reported by other authors [22,23].

Glucose levels in patients with NAFLD were related directly to BMI; as BMI increased, so did average glucose levels, but not significantly so. Some authors like Cabrerizo et al. [24] associated diabetes with NAFLD, a fact demonstrated by the results of this study where 47.5% of the subjects had diabetes, patients in Groups C and D showing the greatest numbers. These findings are compatible with the results of other authors where higher NAFLD prevalence has been reported in diabetics compared to the general population [6,25]. However, a study carried out in South India showed that NAFLD prevalence in diabetics was 56.5%, much higher than the results found here [26]. It is obvious that when subjects have both diabetes mellitus and obesity, the risk of NAFLD increases significantly [27].

Liver function enzyme elevation, particularly alanine aminotransferase (ALT), is often the first sign of NAFLD, an increase of one to three times its normal

value being observed [28]. In this research, even though no statistical significance was found, the most frequent alteration was ALT level elevation (72.5% patients) with a higher level relative to AST level (25% patients), where similarly elevated values for both transaminases were not found. Contrary to this, Gaviria et al. [21] found hypertransaminasemia AST levels (23.3%) more elevated in comparison with ALT (13.3%) in obese patients with NAFLD, which suggests that both enzymes are closely linked to the growing NAFLD-associated obesity epidemic. Studies that have evaluated the etiology of elevated levels of transaminases in the general population of the USA have proposed that elevated levels of ALT, as well as AST, are predictors of the presence of NAFLD [29].

Another abnormality associated with the onset of NAFLD is the elevated levels of alkaline phosphatase. In this regard, a slight increase in levels in 45% total population studied was found, but it was not enough as to be statistically significant when levels between the study groups were compared. These findings are compatible with the results of other authors [1].

There is evidence that patients with hypertriglyceridemia or dyslipidemia have a 5 to 6 fold increased risk of developing NAFLD with respect to the normal population [3,30]. Rousch et al. [1], Martínez et al. [18] and Cabrerizo et al. [24] agree that the foremost factor in NAFLD prevalence is dyslipidemia. In this study, the most characteristic lipid alterations were the significant elevation of triglyceride levels (85%) and cholesterol (82.5%), biochemical parameters that exhibited a significant difference when compared between study groups. This finding leads to the assumption that lipid regulation, synthesis, and metabolism are altered in this group of patients. This abnormality may be due to an adjustment to the rise of protein binding to the sterol regulatory element SREP-1 c, a transcription factor of some genes involved in the de novo synthesis of fatty acids; this element inhibits the oxidation of free fatty acids and the stimulation of the fat content in the liver [31-33]. Similarly, the sterol regulatory element SREBP-2 and low-density lipoprotein (LDL) receptors are regulated downwards in subjects with NAFLD, thus inhibiting cholesterol absorption and the synthesis of very high-density lipoprotein (VLDL) in hepatocytes and resulting in a high triglyceride content in the liver [32]. On the other hand, elevated TG levels can alter the lipid profile even more by reducing the cholesterol in lipoproteins of high density (HDL-C) and by increasing dense LDL particles [34]. In addition, a comparison of BMI between study groups revealed a significant difference among them (Group D>C>B>A). Therefore it can be said that BMI is a factor that predisposes people to NAFLD as it is directly associated with obesity. Data from the NHANES-III study on dyslipidemia also reflect a higher prevalence as BMI increases, especially in men.

In clinical practice, it has been reported that serum bilirubin level is related to various diseases. In this study group, total bilirubin levels were within the normal range, without statistical significance. So far the literature had excluded bilirubin as a guiding test for NAFLD. However, a study by Chang et al. [35] revealed that serum conjugated bilirubin levels were significantly associated with a lower incidence of NAFLD. Nevertheless, some authors found that total bilirubin levels are associated in a negative way [36], and still others observed that unconjugated bilirubin levels are rigorously related to NAFLD [37,38], therefore, it is not clear which bilirubin influences NAFLD presence. Unfortunately, in this study, both bilirubins were not measured. It is clear that more research is needed to elucidate the mechanisms underlying this association and to establish the role of bilirubin as a risk marker of non-alcoholic fatty liver disease.

Some limitations of our study deserve to be commented on. Ultrasound diagnosed Non-alcoholic fatty liver disease without histological confirmation of a fatty liver. A more robust sample could allow other factors associated with NAFLD in obese adults to be identified. In addition, the study population had moderate to severe prevalence of obesity and resistance to insulin was not determined; this has a great influence on the physiopathological process, where tumor necrosis factor (TNF) has been shown to increase in the serum of insulin resistant people, type 2 diabetics or android obesity carriers. Recent studies provide new evidence showing that exposure to tobacco smoke and indirect smoke aspiration can accelerate the development of experimental non-alcoholic fatty liver disease [39,40]. Therefore, future research is needed to establish with precision the pathological condition of the liver. However, our sample was representative where there is evidence of similarities with results published in the international literature.

Conclusion

Non-alcoholic fatty liver disease (NAFLD) includes patients who do not have a history of substantial alcohol use. It is characterized by the accumulation in the liver of macro vesicular fat. Among the most relevant findings was that 82.5% of the patients evaluated in this study presented some obesity and the frequency of NAFLD in relation to gender was similar to male

and female. Glucose levels were related directly to BMI; as BMI increased so did average glucose values. The alterations in the sick population were elevation in ALT levels, higher than AST levels. A slight increase in ALP levels was observed, as well as significant elevation of triglyceride and cholesterol levels. Therefore, the influence of factors such as gender, BMI, and dyslipidemia that reaffirm the incidence of NAFLD in the population studied cannot be ruled out at the onset of the disease. Since the early detection is needed for timely management and to avoid development into cirrhosis and its complications.

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Competing and Conflicting Interests

The authors stated that they have no conflict of interest regarding the publication of this article.

Author Contributions

We are the team of this research. All authors were involved in literature search, experimental work, and the preparation of manuscript. Noé López-Amador was involved in the study design and the selection of patients; Cirilo Nolasco-Hipólito carried out the statistical analysis of the study; Macario de J Rojas-Jimeno carried out the experiments; Octavio Carvajal-Zarrabal wrote the manuscript, supervised, revised and was involved in the comments made on the draft manuscript. All the authors read and approved the final manuscript.

Executive summary

Background: Up to the present, NAFLD diagnosis has been established through the invasive method of biopsy. The search for a non-invasive alternative, for example biomarkers, is a motive for research. Previous studies have shown that for diabetic or obese patients with NAFLD, the profile of liver enzymes as NAFLD biomarkers has so far not defined pathological liver condition as such. This study aim was comparatively analyze the results of previous research on liver function enzymes of obese patients with NAFLD and to corroborate them with this type of patient in Veracruz.

Methods and findings: Forty hospital patients were recruited and classified according to their body mass index (BMI) into 4 groups: Group A (Overweight), Group B (Grade I obesity), Group C (Grade II obesity) and Group D (Grade III obesity), leaving aside those who had a history of hepatitis or consumption of alcoholic beverages. A survey was conducted on these patients to determine sex, signs of liver disease, medicine consumption; laboratory studies which included glucose, total cholesterol, triglycerides, alanine aminotranferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), total protein, and total bilirrubin were also determined. Glucose levels were directly related to BMI, and the most frequent disorder was elevation of ALT levels (72.5%), higher than AST (25%) and ALP (45%). Some variables of the lipid profile showed a significant ($P<0.05$) elevation of triglycerides (85%) and highly significant ($P<0.01$) raised cholesterol levels (82.5%).

Conclusions: The study shows that there are alterations in liver enzymes levels and highlight the importance of gender, BMI and dyslipidemia to assess the risk of individuals with NAFLD, which reaffirm the association with the disease.

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