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Identification of non-alcoholic fatty liver disease in Saudi females and validation of non–invasive indices

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ABSTRACT

Background: There is a need to identify cost-effective, simple, non-invasive diagnostic tools for Non Alcoholic Fatty Liver Disease (NAFLD), in high risk communities, as an alternative to the invasive and costly methods. The objectives of this study were to study the prevalence of NAFLD using ultrasound among female students in Hail, Saudi Arabia and using these results to validate different simple non-invasive indices. *Methods:* A total of 568 females, 20-30 years of age, were enrolled. Ultrasound tests were performed to identify different grades of NAFLD. An age-matched case (NAFLD) and control (healthy) groups (105 participants, each), according to the ultrasound results, were compared to identify risk factors for NAFLD. Regression models were used to examine the associations between NAFLD and potential risk factors. Validation of different non-invasive indices against ultrasound results were conducted using Receiver Operating Characteristic (ROC) Curve cut offs. *Results:* The prevalence of mild NAFLD was 15.5% and moderate NAFLD was 4.9%, while no severe NAFLD cases were observed. The percentage of participants in the case group who had body mass index (BMI) ≥ 30, impaired fasting glucose, aspartate aminotransferase over alanine aminotransferase, [AST/ALT] ≥0.8, was higher ($p<0.001$) than that of the control group. AFLD fibrosis score showed the highest sensitivity (96%) for the diagnosis of NAFLD, while the highest specificity was for the HAIR score (89.7%). *Conclusions:* Prevalence of NAFLD among young females in Hail was high. NAFLD fibrosis score showed the highest accuracy among the non-invasive methods in the diagnosis of NAFLD.

Keywords: Non-Alcoholic Fatty Liver Disease, Young Saudi Females, Prevalence, Ultrasound, Non-invasive methods

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a silent disease that is characterized by having steatosis in ≥5–10% of the hepatocytes with the absence of alcohol consumption or any other aetiologies of liver disease (Younossi et al., 2016). A series of hepatic pathologies may occur because of NAFLD, which might include the relatively benign steatosis that may progress to more severe conditions such as non-alcoholic steatohepatitis, cirrhosis and hepatocarcinoma (Brunt, 2010). Because of the escalating prevalence of its risk factors, NAFLD is increasingly becoming an important public health concern (Chalasani et al., 2012). With rising epidemics of worldwide obesity and metabolic syndrome (MS), the prevalence of NAFLD is expected to increase. Studies suggested a strong association between NAFLD and MS, including obesity, hypertension, diabetes, and dyslipidemia (Vanni et al., 2010, Chang et al., 2013). Previous studies showed that the prevalence of NAFLD varied from 16% in normal weight individuals without the presence of metabolic risk factos, to a range of 43-60% in patients with diabetes (Williamson et al., 2011), and even could rise up to 90% in patients with hyperdyslipidemia (Gaggini et al., 2013).

In recent years, an increasing number of Saudis are suffering from MS and other risk factors of NAFLD. Previous studies showed that the prevalence of NAFLD in Saudi Arabia was 7% to 10% (El-Hassan et al., 1992, Al-Quorain et al., 1994, Akbar et al., 2003). Although many previous studies of NAFLD in Saudi Arabia identified the risk factors that resulted in high prevalence of NAFLD to include diabetes, obesity, and hyperlipidemia, no recent epidemiological studies have measured the NAFLD disease burden (Al-hamoudi et al., 2012). In addition, the differences in diagnostic measurements among these studies suggested that the prevalence of NAFLD in Saudi Arabia was possibly underreported. This might indicate a need for nation-wide studies to assess NAFLD prevalence in the Saudi population.

The accuracy of NAFLD diagnosis is strongly dependent on the diagnostic tool (Vernon et al., 2011). Liver biopsy is considered the gold standard for the diagnosis of NAFLD. Nevertheless, since it is an invasive method and due to its high cost and potential risk of mortality and morbidity, its use is not advised for large-scale population studies. Guidelines suggested the use of liver biopsies to be limited for only those who would benefit the most from the diagnostic, therapeutic guidance, and prognostic perspectives (Chalasani et al., 2012). Therefore, there is a need to identify cost-effective, simple, and non-invasive diagnostic tools of NAFLD. Currently, the prominent non–invasive techniques for population-based studies include the use of a combination of serum biomarkers with other non-invasive methods such as ultrasound, scintigraphy, or magnetic resonance imaging (Schwenzer et al., 2009). The promising development of various indices such as HAIR score (hypertension, ALT and insulin resistance), NAFLD fibrosis score, hepatic steatosis index (HSI) and fatty liver index (FLI), combine a number of biomarkers into the diagnostic panel with improved sensitivity and specificity (Estep et al., 2010). However, these indices need validation against standard, non-invasive, diagnostic tools, such as ultrasound, across different populations to show the ability to unequivocally diagnose and stage NAFLD, while excluding other conditions. The objectives of this study were: (i) to study the prevalence of NAFLD among female university

students in the Hail region, Saudi Arabia, (ii) to establish the risk factors associated with the prevalence of NAFLD in the study group, and (iii) to validate different simple, non-invasive indices, which can be of clinical importance.

2. MATERIALS AND METHODS

Subjects and study design

A cross-sectional study was conducted at the female campus of the University of Hail from September 2016 until March 2017. Data were collected by recruiting 568 female students (after cleaning and removing of missing data) to study the prevalence of NAFLD among female students. Inclusion criteria were females between the age 20 and 30 years, after voluntarily signing a written informed consent form. The exclusion criteria were: (i) females with alcohol history, (ii) presence of a chronic liver disease, (iii) presence of hepatitis B virus surface antigen or the presence of hepatitis C virus antibodies, (iv) subjects with evidence of any disease related to chronic inflammation, oxidative stress, hydric imbalance, nutrient absorption, or nutrient metabolism, (v) participants using chronic drugs or dietetic treatments up to 6 months before participation in this study, and (vi) acute illness at the time of data collection. The participants filled a closed pretested questionnaire that consisted of two parts: (i) demographic information and (ii) medical history. Ethical approval was obtained from the University of Hail Research Ethics following the rules of the Helsinki Declaration (Approval number 28-2016). The questionnaire was validated by collecting the results of a pilot study that consisted of 120 samples. Cronbach alpha test was performed of the subsample and was found to be 0.72. Data were cleaned for inconsistencies and outliers, and then missing data (n=19) were removed from the original sample.

Data collection

Dyslipidemia and arterial hypertension were checked using appropriate measurements. Arterial hypertension was determined by testing blood pressure (BP) with validated automatic BP measurement machine (BP A7 TOUCH, Microlife USA, Dunedin, Florida, USA) with subjects in resting position. Two measurements were carried out separated by two minutes, and the average was taken. Overnight fasting for blood tests of platelets count from the complete blood count and blood chemistry (ALT, AST, Albumin, GGT [Gamma-glutamyl transferase], cholesterol, triglycerides (TG), HDL, LDL, fasting blood glucose [FBG]) were performed for all subjects at Hail General Hospital laboratories (Siemens Healthcare Diagnostics Inc., Newark, DE, USA), according to the standard procedures. Impaired fasting glucose (IFG) was defined when overnight fasting blood glucose is > 100 mg/dl.

Ultrasound tests were performed for participants to identify different grades of NAFLD at the University of Hail clinics (Siemens Acuson S2000, Siemens Medical Solutions, Mountain View, CA, USA). The degree of fatty infiltration of the liver was evaluated by using the conventional grading system, normally used by radiologists (Saadeh et al., 2002, Hernaez et al., 2011, Mottin et al., 2004). The non-invasive methods to diagnose NAFLD were determined according to Dixon et al. (2001) for the HAIR score, Angulo et al. (2007) for NAFLD fibrosis score, Lee et al. (2010) for HSI and Bedogni et al. (2006) for FLI. NAFLD was defined as the presence of fatty liver disease by ultrasound in the absence of a potential cause of chronic liver disease according to Lee et al. (2010). NAFLD was graded according to the fat filtration in the liver, mild NAFLD involves less than 30% hepatocytes (fibrosis stage 0-1, while moderate NAFLD up to 60% (fibrosis stage 1-3), and severe NAFLD above 60% (fibrosis stage 3-4, Ploeg et al., 1993).

Case-control selection

For every case participant, an age-matched control participant was selected (± 1 year). Control samples were defined as participants who had normal ultrasound results. However, the case group was defined as the ones who had manifestations of NAFLD according to the ultrasound results for hepatic screening. A subsample of 105 high-risk profile subjects along with 105 age-matched low-risk profile subjects were selected in the nested case-control study. Cases and controls were compared to identify risk factors for NAFLD. Results were then compared with ultrasound results to identify the most sensitive indicator for the female subjects in the Hail region of Saudi Arabia suffering from NAFLD.

Data analysis

The Statistical Package for Social Sciences (version 20, SPSS, Statistic Package for Social Sciences, IBM Corp., Armonk, NY, USA) was used for data analysis. Descriptive data were expressed as means ± standard deviations (SD) and percentages as appropriate for quantitative and qualitative data. Normality was checked by Kolmogorov-Smirnov test. For the nested case-control study, P-value was determined by Mann-Whitney U test. Furthermore, bivariate and multivariate analyses were performed by means of a logistic regression model to identify risk factors that are predictive of advanced stages of NAFLD by calculating the odds ratio (OR) and 95% confidence interval (CI) for different risk factors. Receiver Operating Characteristic (ROC) curve analysis was used to identify

appropriate cut offs for each score to identify NAFLD with the highest sensitivity and specificity applicable to the selected female Saudi sample.

3. RESULTS

The prevalence study

The clinical and biochemical data of the subjects were illustrated in Table 1. The mean age of the study group was 22.6 years, with a mean body mass index (BMI) of 26.2, which is considered slightly overweight. However, other biochemical values were considered normal for the study group such as ALT (21.9 U/l), AST (34.8 U/l), AST/ALT ratio (0.66), albumin (44.1 g/l), GGT (20.9 U/l), platelets (265.3 x 10^9/l), cholesterol (4.1 mmol/l), triglycerides (0.94 mmol/l), HDL (1.3 mmol/l), LDL (2.6 mmol/l), fasting blood glucose (5.1 mmol/l), SBP (109.6 mm Hg), and DBP (71.9 mm Hg).

Ultrasound results showed that 15.5% of the subjects were identified with mild NAFLD, whereas 4.9% were identified with moderate NAFLD. However, no marked severe cases were identified in the study group (Figure 1). Using the non-invasive index scores to predict the prevalence of NAFLD among the study group, it has been shown that HAIR score identified 14.4%, NAFLD fibrosis score predicted 19.5%, HSI predicted 16.4, while FLI could identify 11.6 % of the study subjects as cases (Figure 2).

Table 1 Clinical and biochemical data of subjects.

Parameter	Mean	SD[1]	Normal Value
Age (years)	22.6	4.7	-
Height (cm)	157.1	6.04	-
Weight (kg)	64.6	15.9	-
BMI (kg/m^2)	26.2	6.3	18.5-24.9
ALT (U/l)	21.9	8.5	≤30
AST (U/l)	34.8	17.8	10-40
AST/ALT ratio	0.66	0.21	<0.8
Albumin (g/L)	44.1	3.8	35-55
GGT (U/L)	20.9	6.7	0-45
Platelets (x 10^9/L)	265.3	68.5	150-400
Cholesterol (mmol/L)	4.10	0.78	<5.2
Triglycerides (mmol/L)	0.94	0.55	<1.7
HDL (mmol/L)	1.3	0.3	>1.0
LDL (mmol/L)	2.6	0.7	2.59-3.34
FBG (mmol/L)	5.1	0.6	<6.1
SBP (mm Hg)	109.6	14.3	<120
DBP (mm Hg)	71.9	10.7	<80

Abbreviations: SD: Standard deviation, BMI: Body mass index, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, FBG: Fasting blood glucose, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

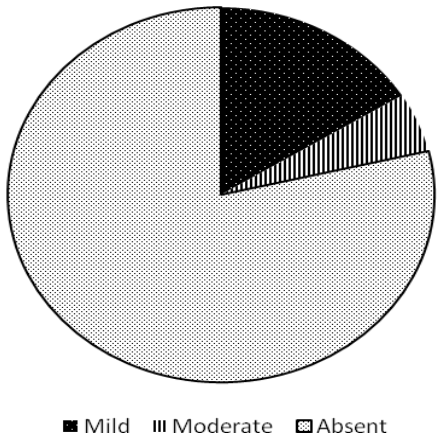

Figure 1 NAFLD prevalence of the study sample as identified by ultrasound

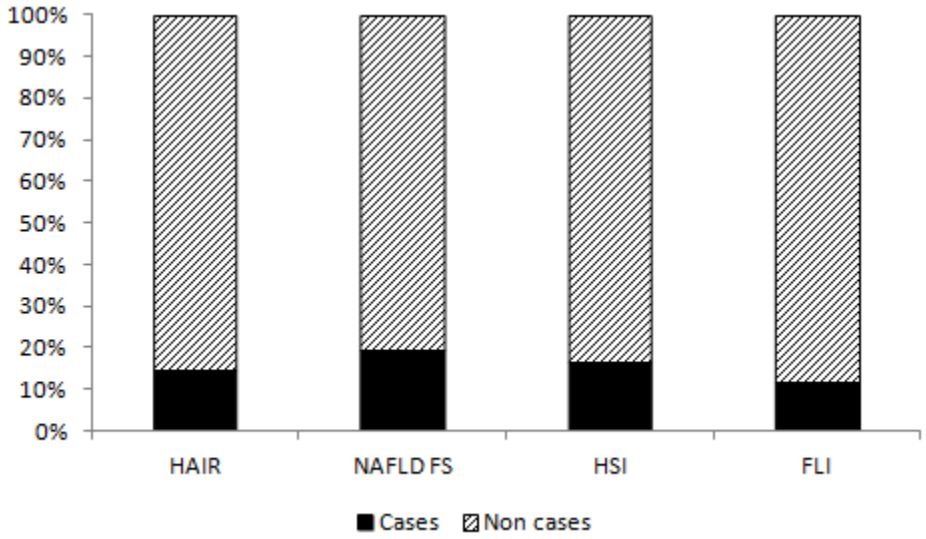

Figure 2 Case identification of NAFLD in the nested case-control experiment, using non-invasive scores.
Abbreviations: HAIR: hypertension, ALT and insulin resistance; NAFLD FS: Non-Alcoholic Fatty Liver Disease fibroses score; HSI: hepatic steatosis index; FLI; fatty liver index.

The case-control study

In the nested case-control study (Table 2), results revealed that the percentage of participants in the case group who had BMI≥30 (57.6%) was higher ($p<0.001$) than that of the control group (18.3%), with a positive association between BMI and NAFLD (OR=3.9, 95% CI = 1.8-9.8). The percentages of participants who had IFG in the case group (45.7%) was higher ($p=0.01$) than that of the control group (20.1%), with a positive association between impaired fasting glucose (IFG) and NAFLD (OR=2.9, 95% CI = 1.1-6.5). Although the percentage of participants in the case group who had high blood pressure (HBP, 22.5%) was higher ($p=0.01$) than that of the control group (6.2%), however, this was not shown to have an association between HBP and NAFLD (OR=1.12, CI=0.9-1.9). The percentage of participants in the study group who had AST/ALT ≥ 0.8 (39.3%) was higher ($p<0.001$) than that of the control group (12.2%), with a positive association between AST/ALT ratio and NAFLD (Table 2). Mean values of BMI (kg/m^2), AST (U/l), and AST/ALT in the case group (32.2, 41.3, and 0.78, respectively) was higher ($p<0.01$) than that of the control group (24.7, 33.2, and 0.66, respectively).

According to the AUC, all the non-invasive scores studied showed acceptable sensitivity and specificity for predicting NAFLD compared with the ultrasound results (Table 3). The highest sensitivity was for the NAFLD fibrosis score (96.0%), while the highest specificity was for the HAIR score (89.7%; Figure 3). The OR of the non-invasive scores in predicting the NAFLD ranged from 2.8 to

12.8. The NAFD fibrosis score had the highest OR for predicting NAFLD compared with ultrasound (OR = 12.8, CI=7.9–9.2, p<0.001), followed by the HSI (OR = 9.8, CI=9.4–11.1, p<0.001).

Table 2 Comparison of selected clinical and biochemical parameters between case and control groups

Parameters	Cases	Controls	P-Value	OR (95 % CI)
Age (years)	22.9 ± 2.8	22.7 ± 2.6	0.76	-
BMI (kg/m2)	32.3 ± 3.4	24.7 ± 6.4	0.0013	-
ALT (U/L)	25.9 ± 6.4	24.9 ± 7.2	0.25	-
AST (U/L)	41.3 ±19.5	33.3 ± 18.2	0.0004	-
AST/ALT ratio	0.78 ± 0.40	0.66 ± 0.29	0.001	-
BMI (≥30 kg/m^2) (%)	57.6	18.3	0.0003	3.9 (1.8 -9.8)*
IFG (%)	45.7	20.1	0.011	2.9 (1.1-6.5)*
HBP (%)	22.5	6.2	0.012	1.1 (0.9-1.9)
AST/ALT ratio ≥0.8 (%)	39.3	12.2	0.0002	6.6 (4.4-11.3)*

* Significant result of OR at p<0.05

IFG: Impaired fasting glucose

OR was determined by bivariate logistic regression

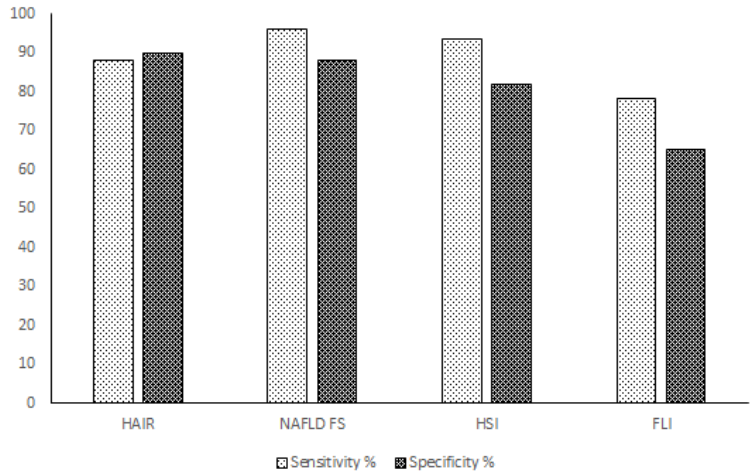

Figure 3 Sensitivity and specificity percentages of the different non-invasive scores in predicting NAFLD cases compared with ultrasound.

Abbreviations: HAIR: hypertension, ALT and insulin resistance; NAFLD FS: Non-Alcoholic Fatty Liver Disease fibroses score; HSI: hepatic steatosis index; FLI; fatty liver index.

Table 3 Accuracy of non-invasive scores for predicting NAFLD as compared to ultrasound

Parameter	Sensitivity %	Specificity %	AUC
HAIR	88.0	89.7	0.85 (p<0.0001) OR=8.4 95%CI= 7.9–9.2
NAFLD FS	96.0	87.9	0.82 (p<0.0001) OR=12.8 95%CI=12.4–19.7
HSI	93.5	81.7	0.72 (p<0.0001) OR=9.8 95%CI=9.4–11.1

			0.52(p<0.024)
FLI	78.1	65.1	OR=2.8
			95%CI=2.4–5.7

AUC: Area Under the Curve

OR was determined by multivariate logistic regression

Abbreviations: HAIR: hypertension, ALT and insulin resistance; NAFLD FS: Non-Alcoholic Fatty Liver Disease fibroses score; HSI: hepatic steatosis index; FLI; fatty liver index.

4. DISCUSSION

Identifying the prevalence of NAFLD, especially among young populations, has prognostic significance, which might help in avoiding the progression to the advanced fibrosis conditions. Although liver biopsy is the best and the most accurate procedure to diagnose NAFLD, however, it is a costly and a highly invasive technique, which might subject a threat to the patient's general health. The use of ultrasound has been proposed as a non-invasive alternative (Hernaez et al., 2011), however, it is not a cost-effective technique, especially for screening tests. Therefore, there is a need to identify simple, non-invasive diagnostic methods, with high sensitivity and specificity, to predict NAFLD in patients, as well as using these methods in epidemiological studies for large-scale populations. The most popular non-invasive scores, which have been developed by various researchers, include HAIR score, NAFLD fibrosis score, HSI and FLI (Dixon et al., 2001, Angulo et al., 2007, Lee et al., 2010, Bedogni et al., 2006). However, these scores were not validated in the Saudi population. Therefore, the present study was conducted to validate these scores in Saudi young female population in comparison with ultrasound results.

Our results showed that the prevalence of NAFLD among the study group was higher than the previous studies of adults in Saudi Arabia. Using the ultrasound, our study identified 20.4% of the sample to have either mild or moderate NAFLD. However, other studies (El-Hassan et al., 1992, Al-Quorain et al., 1994, Akbar et al., 2003) showed that the prevalence of NAFLD in Saudi Arabia was between 7-10%. In another prospective study among hospital patients referred for ultrasound in Saudi Arabia, Al-hamoudi et al. (2012) showed that the prevalence of NAFLD was 16%. The higher percentage of NAFLD among our youthful female participants compared with other studies in Saudi Arabia could be due to the prevalence of several risk factors of NAFLD in the Hail region of Saudi Arabia.

It has been shown that the prevalence of obesity among adults of the Hail region was 33.9%, which ranked the highest among the other regions of Saudi Arabia (Al-Othaimeen et al., 2007). Obesity is considered the most important risk factor for NAFLD (Hernaez et al., 2011). Other risk factors of NAFLD were shown to be present in the Hail region. These include MS, increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels (Grundy et al., 2004). Specifically, there is a striking increase in the prevalence of DM2 in Saudi Arabia throughout the last 4 decades. In the late 80s of the pervious century, only 4.3% of adults suffered from DM2 according to Fatani et al. (1987). However, in the late 90s of the previous century, Al-Nuaim et al. (1996) indicated that adults suffering from DM2 was between 7-14%, depending on gender and livelihood. Later studies showed that the prevalence of DM2 in Saudi Arabia increased to 23.7% according to Al-Nozha et al. (2004) and 25.4%, according to Al-Rubeaan et al. (2014). The prevalence of DM2 in adults in the Northern area of KSA, including Hail region, was the highest (27.9%) among other regions of Saudi Arabia (Al-Nozha et al., 2004). Furthermore, another study (Al-Nozha et al., 2005) suggested high levels of MS in Saudi Arabia, especially among adult females (42%). While a current study by Al-Rubeaan et al. (2018) showed that the prevalence of MS among female adults of Saudi Arabia was 35.4%. The higher percentages ($p<0.01$) of BMI (≥30 kg/m2), IFG and HBP of the case group compared with the control group (Table 3) also emphasized the effect of the different risk factors of MS on increasing the prevalence of NAFLD. The results of this study were in agreement with other studies (Ong et al., 2005, Uslusoy et al., 2011, Cichoż-Lach et al., 2012), which showed that younger population suffering from obesity (BMI>30), could be considered at a higher risk for MS as well as mild and moderate risk of NAFLD. Another risk factor of NAFLD is the presence of single nucleotide polymorphism, namely rs738409 allele (Ile148Met) in PNPLA3 gene that would increase the risk of NAFLD. This allele was found in nomadic Bedouins of some Arabian Peninsula populations (John et al., 2015). Many Bedouin tribes are extended along the Arabian Peninsula, including Saudi Arabia, which might result in the presence of rs738409 allele in Hail region.

The use of several biomarkers, such as ALT, AST and GGT has been suggested for the diagnosis of NAFLD. However, 78% of NAFLD patients could have normal liver enzymes (Browning et al., 2004, Torres et al., 2008). AST level in the case group was higher ($p< 0.001$) than the control group (41.3 vs. 33.3 U/l, respectively). Nevertheless, the AST level of the case group was slightly more than the upper levels of AST. In addition, there was no differences ($p>0.05$) in ALT levels in both groups. The lack of sensitivity of these biomarkers, might suggest the importance of finding alternative non-invasive methods that would reflect better diagnosis of

NAFLD. The ratio of AST/ALT is another commonly used biomarker method in the diagnosis of NAFLD (Castera et al., 2013). AST/ALT ≥0.8 is considered a risk factor for NAFLD, while an AST/ALT ratio > 2 suggests alcoholic hepatitis (Matteoni et al., 1999). Indeed, the percentage of females in the case group with AST/ALT ≥0.8 was higher (p=0.001) than that in the control group (Table 2). However, the average AST/ALT ratio within each group was under the suggested upper level of ≥0.8. In addition, since both AST and ALT level are not sensitive for the diagnosis of NAFLD, this might caution the use of AST/ALT as a biomarker for the diagnosis of NAFLD.

To our knowledge, this is the first study that tested the sensitivity and specificity of four non-invasive scores in comparison with ultrasound results to identify the best non-invasive method to be used in the diagnosis of NAFLD in Saudi Arabia. Our study showed that both NAFLD fibrosis score and HSI has relatively high sensitivity in predicting cases in comparison with ultrasound results. The HAIR score was relatively specific but not as sensitive as the former two scores. Different studies validated some non-invasive scores, however, NAFLD fibrosis score remained the most validated test (Brunt et al., 2015). A study by Wong et al. (2008) found out that the NAFLD fibrosis score showed high sensitivity to the diagnosis of NAFLD and that 79% of liver biopsies could be avoided by the use of the NAFLD fibrosis score in the Chinese population. However, the later study showed that HAIR score was not significant for patients with or without advanced fibrosis. Results of our study were in agreement with Angulo et al. (2002) who showed that NAFLD fibrosis score had the highest predictability among other non-invasive techniques used.

5. CONCLUSIONS

In conclusion, results of our study revealed that the prevalence of mild NAFLD in Saudi young women was around 15.5%, while the prevalence of moderate NAFLD was 4.9%, which is considered higher than the prevalence studies found in other studies. However, no server cases identified using ultrasound. Obesity, IFG and AST/ALT≥0.8 were the highest risk factors in the Saudi young females with NAFLD. NAFLD fibrosis score showed the highest sensitivity (96%) for the diagnosis of NAFLD among females in the Hail region of Saudi Arabia, while the highest specificity was for the HAIR score (89.7%). There is a need to test the presence of the allele rs738409 as an independent risk factor of NAFLD, especially in the Northern regions of Saudi Arabia.

Author Contributions

AYA conceived and designed the study, writing of the original draft. SE and FSA analyzed and interpreted data. SE, SQ, MMA, SB, RB, JAC, NAA conducted research, provided research materials, and collected and organized data. SS conducted blood chemistry tests and analysis. AYA, FSA, SE wrote initial and final draft of the article. All authors have critically reviewed and approved the final draft of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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