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A Study on The Proportion/Hospital Based Prevalance of Non-Alcoholic Fatty Liver Disease (NAFLD) in Diagnosed Cases of Polycystic Ovarian Syndrome (PCOS) Seen in A Tertiary Care Centre of Bihar

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1. Abstract

1.1. Aims

To evaluate the proportion/hospital-based prevalence of non-alcoholic fatty liver disease in diagnosed cases of polycystic ovarian syndrome and to evaluate and compare the various relevant biochemical parameters and their derangements in cases of PCOS with NAFLD and cases of PCOS without NAFLD.

1.2. Methods

This was a cross-sectional observational study which included 86 patients of PCOS in the age group of 15-35 years between December 2020 to January 2021.A detailed history was obtained from cases. Patients were screened for clinical signs of hyperandrogenism. Transabdominal USG was undertaken to detect polycystic ovaries and for detecting steatosis and its grade. Also, shear wave elastography was performed for assessing the liver fibrosis and the reading noted. Anthropometric measurement and Hormone assay were done. All the data were analysed using SPSS package for windows.

1.3. Results

Out of 86 PCOS patients, 36 patients had NAFLD (41.87%) and 50 patients had no NAFLD (58.13%). Overweight patients have 27 times more risk of developing NAFLD condition (OR 27.429, 95% CI 5.69-132.27), whereas Obese patients have 36 times risk (OR 36.00, 95% CI 5.65-229.29) of developing NAFLD condition. Higher BMI is associated with developing of NAFLD. Waist/Hip ratio of ≥0.8 was also noted to have 89 times more risk of developing NAFLD (OR 89, 95% CI 17.767-448.341). Higher testosterone levels were associated with 1.35 times more risk of developing NAFLD (OR 1.352, 95% CI 0.552-3.311). Higher OGTT values were associated with 1.292 times more risk of developing NAFLD. Deranged lipid profile was found to confer more risk of developing NAFLD.

1.4. Conclusion

NAFLD is commonly found in women with PCOS, and may be an early sign of metabolic syndrome. Patients with PCOS should be screened for NAFLD as early interventions can be an important measure to reduce the risk of progression to advanced liver disease.

2. Introduction

The term polycystic ovarian syndrome (PCOS) was first described by Irving Stein and Michael Leventhal as a triad of amenorrhoea, obesity and hirsutism in 1935 when they observed the relationship between obesity and reproductive disorders [1]. It is hence also known as Stein Leventhal syndrome or hyperandrogenic anovulation and is the most common endocrine ovarian disorder affecting approximately 2 to 8 percent women of reproductive age group worldwide [2]. The connection between PCOS and non-alcoholic fatty liver disease (NAFLD) was first reported in 2005 and subsequent retrospective studies have confirmed this association [3]. Gambarin-Gelwan et al demonstrated hepatic steatosis in 55% of the 88 PCOS women in their retrospective study [3]. The first study to include a control group was a prospective study of Chilean women with PCOS by Cerda et al [4]. Recent studies have demonstrated an association between PCOS and NAFLD [5-14]. Between 30 to 40 percent of PCOS women have NAFLD on imaging which may relate to their increased metabolic co morbidities [2]. NAFLD occurs as a result of abnormal lipid handling by the liver which sensitizes the liver to injury and inflammation. It can progress to NASH (non-alcoholic steato-hepatitis) which is characterised by hepatocyte injury and apoptosis. With time and further inflammation NASH can progress to cirrhosis [2]. Thus, given the young age at which NAFLD can occur in PCOS, these women may be at significant risk for progressive hepatic injury over the course of their lives. As lifestyle and medical intervention can help to improve hepatic fibrosis, proper and early diagnosis and referral for treatment are of critical importance. This study was aimed to evaluate the proportion/ hospital- based prevalence of NAFLD in diagnosed cases of PCOS in a tertiary care centre of Bihar. The objectives of this study were to evaluate the various relevant biochemical parameters and their derangements in PCOS cases and to compare the biochemical parameters in cases of PCOS with NAFLD with cases of PCOS without NAFLD.

2.1. Pathophysiology of NAFLD in PCOS

In women with polycystic ovaries, the peripheral insulin resistance is due to a defect beyond activation of the receptor kinase, specifically leading to reduced tyrosine auto phosphorylation of the insulin receptor [15]. Insulin resistance (IR) is detected in up to 80% of cases of NAFLD and there is a near universal association between NAFLD and IR irrespective of obesity [16]. While the exact pathogenesis of NAFLD is still unclear, evidence supports IR as a primary factor in its development [17]. The “two-hit theory” that was initially suggested about 15 years ago suggested that IR is the first “hit” to cause hepatic steatosis, when insulin resistant visceral adipocytes release free fatty acids that flow to the liver and ac- cumulate; cytokine stresses and apoptosis are the second “hit”, which mediate the progression to NASH [18]. More recently, two additional concepts, “multiple-hit” and “distinct hit” theories, are being considered to explain the pathogenesis of NAFLD/NASH, and both still include IR as an integral

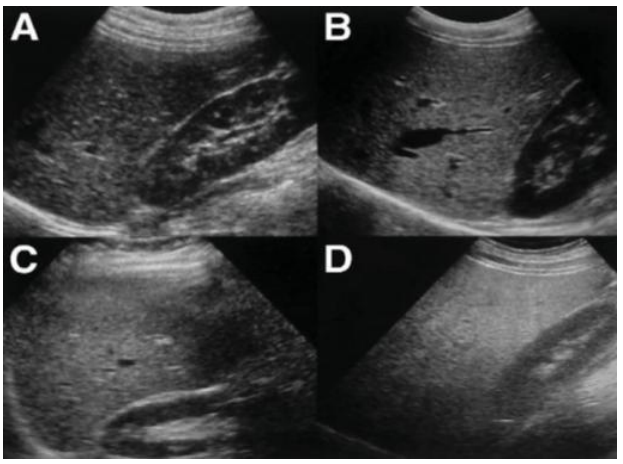

Image 1: Ultrasonographic criteria for grading of hepatic steatosis.

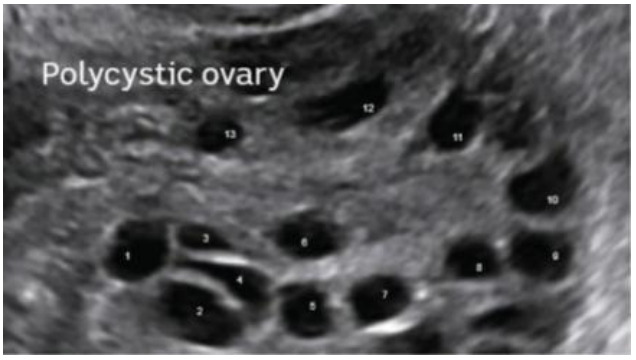

Image 2: Ultrasound image showing polycystic ovary.

Image 3: Region of interest& Readings obtained in shear wave elastography.

component [19]. The "multiple-hit" theory conceptualizes NAFLD/ NASH patho- genesis in the same way as the "two-hit" theory, where NASH is generally a condition preceded histologically by simple steatosis and pathophysiologically by IR and its associated metabolic disturbances [20]. However, fatty infiltration of the liver then leads to a series of parallel multiple hits, such as cytokines, adipokines, and oxidative stresses, which mediate the progression to NASH and fibrosis. The "distinct hit" hypothesis was generated by evidence that NASH and pure fatty liver could arise as two independent conditions, since inflammation occasionally precedes steatosis and patients with NASH may present with very little steatosis [21], therefore according to this model, distinct pathways are activated (potentially by insulin resistance) which lead to either simple steatosis or NASH, rather than an accumulation of multiple parallel hepatotoxic injuries.

3. Materials and Methods

The present study had been undertaken at All India Institute of Medical Sciences, Phulwarisharif, Patna (Bihar). The study period was from December 2020 to January 2021.

3.1. Ethical Consideration

The topic "A study on the proportion/Hospital based Prevalence of Non Alcoholic Fatty Liver Disease (NAFLD) in Diagnosed cases of Polycystic Ovarian Syndrome (PCOS) seen in a Tertiary care centre of Bihar" was duly submitted before the ethical committee of our institution and the ethical committee approval was obtained (AIIMS/ Pat/ IEC/ PG Th/ Jan 19/ 04).

3.2. STUDY DESIGN

Cross sectional study

Study participants: humans only

3.3. Inclusion Criteria

1. Females willing to be part of the study.
2. Diagnosed cases of polycystic ovarian syndrome using Rotterdam criteria (2 out of 3 after excluding other etiologies).

I. Oligo/anovulation (after excluding thyroid disorder, hyperprolactinemia, congenital adrenal hyperplasia).

II. Polycystic ovaries >= 12 follicles (2 to 9 in diameter) in each ovary or ovarian volume >10 cc.

III. Biochemical and /or clinical signs of hyperandrogenism:

1. Biochemical: Total testosterone >70 ng/dl

   Androstenedione> 250 ng/dl

   DHEAS >350 mcg/dl

   Serum free testosterone >6.8pg/ml

   17 hydroxyprogesterone>200ng/dl

2. Clinical: hirsutism, acne, acanthosis nigricans.

3.4. Exclusion Criteria

1. Patients who do not meet the inclusion criteria.
2. Patients meeting the inclusion criteria not willing for study.
3. PCOS with secondary causes of hepatic fat accumulation such as alcohol consumption, long term use of steatogenic medications, hereditary disorders, viral etiologies.
4. Patients with chronic liver disease, chronic renal failure, malignant disease.

Definition of NAFLD: Evidence of hepatic steatosis either by imaging or histology, in the absence of other possible causes of hepatic fat accumulation, such as significant alcohol consumption, use of teratogenic medications, viral infections, hereditary disorders. In our patients NAFLD was diagnosed with the help of real-time ultrasonographic imaging study and also point shear wave elastography was used.

4. Sampling

a. Sampling population: Gynaecological patients visiting the department of obstetrics and gynaecology AIIMS Patna and diagnosed with PCOS.

b. Sample size calculation: In a similar study Qu et al reported a prevalence/proportion of 31%. With 95 % confidence level and 5 % precision, desired sample size was calculated using open epi tool. It was fixed at 162.

c. Sampling technique: convenient/ purposive sampling.

d. Study procedure: Hospital based cross sectional study.

4.1. NAFLD Diagnostic Method Used

Real time ultrasonographic study and shear wave elastography were used to diagnose NAFLD in our study. Shear-wave elastography is an ultrasound-based imaging technique to detect the degree of fibrosis in patients with NAFLD.

5. Methods

This was a cross-sectional observational study which included 86 patients of PCOS in the age group between 15-35 years attending Obstetrics & Gynaecology out-patient department AIIMS PATNA. The study was carried out between December 2020 to January 2021.

5.1. Work Up of Patients

The patients visiting department of obstetrics and gynaecology AIIMS PATNA with complaints of menstrual irregularities – oligomenorrhoea/ hypomenorrhea, weight gain, hirsutism (excess body hair, including the chest, stomach, face and back), acanthosis nigricans (brown to black, poorly defined velvety hyperpigmentation of the skin, may present with thickened, velvety relatively darker areas of skin on the neck, armpits and in skin folds), acne or oily skin, infertility and other symptoms suggestive of PCOS was subjected to detailed history. The PCOS patients were selected based on the Rotterdam's 2003 criteria. After fulfilling the selection criteria, all women were briefed and counselled about the study and informed written consent was obtained. PCOS diagnosis was made according to the 2003 Rotterdam criteria. We considered oligo-anovulation by the duration of the cycles of 35 days or more. Clinical hyperandrogenism was defined as the presence of hirsutism, acne, androgenic alopecia, or virilization, and was considered in those with as score > 8 on the modified Ferriman-Gallwey scale, obtained in the initial evaluation. Patients were excluded if they had any of the following exclusion criteria: 1) other causes of irregular menstrual cycles or androgen excess including hyperprolactinemia, uncontrolled thyroid disease, congenital adrenal hyperplasia, premature ovarian failure, Cushing's syndrome, androgen-secreting tumour, or pregnancy; 2) history of known liver disease or other medical problem thought to cause an elevation in liver enzymes; 3) history of medication use clinically thought to cause an elevation in liver enzymes; or 4) history of significant alcohol consumption, defined as greater than one alcoholic beverage per day ,5) renal disease ,malignancy etc. A detailed history was obtained from cases for intake of any hormonal drugs, including OCP as well as medication for lowering blood pressure, blood lipids and glucose. Menstrual history in detail was taken. Patients were screened for clinical signs of hyperandrogenism (acne, oily skin and hirsutism). Clinical hyperandrogenism was defined using a modified Ferriman-Gallaway (FG) score for evaluating and quantifying hirsutism in women using nine body areas (upper lip, chin, chest, upper and lower abdomen, thighs, upper and lower back and upper arm). Hair growth was rated from 0 (no growth of terminal hair) to 4 (extensive hair growth) in each of the nine locations. A score >/= 8 was indicative of androgen excess. Transabdominal USG was undertaken to detect polycystic ovaries. The volume and the number of small follicles (measuring 2–9 mm in diameter) of each ovary were determined. Transabdominal ultrasound was also done for detecting steatosis and its grade. Also, shear wave elastography was performed for assessing the liver fibrosis and the reading noted. Anthropometric measurement of the cases was taken. Height in cm (bare foot, standing erect against wall), weight in kg (in a weighing scale, bare foot, light clothing), waist circumference in cm (minimum circumference at the waist level), and hip circumference in cm (maximum circumference at the level of buttocks) was measured by principal investigator in all the participants included in the study. Body mass index (BMI) was calculated by the formula: body weight (kg)/height in metre squared. Patient was allowed to drink 75gm glucose solution and blood was drawn after two hours i.e. 75grams glucose tolerance test (GTT), <140 mg/dl was considered normal, 140-199 mg/dl as impaired sugars, ≥ 200 mg/dl as diabetic. Obesity was assessed according to WHO criteria as a body mass index (BMI). Calculated as BMI=weight in kilogram /height in metre squared. Classified as <18.5: Underweight, 18.5-24.9: Normal, 25-29.9: Overweight, 30-34.9: Obese, ≥35 morbid obese. Body fat distribution was assessed by measurements of the waist to hip girth ratio (WHR). A WHR <0.85 was considered normal. Hormone assay was done in the early follicular phase and samples were taken on day 2 of menstruation for all women. Follicle stimulating hormone (FSH), luteinising hormone (LH), Serum-prolactin, total testosterone, Lipid profile (LP) was done. The normal cutoff for FSH -10.2 mIU/ml in the follicular phase and for LH <12.5mIU/ml was taken. A prolactin level <29.2 ng/ml was taken as normal, a total testosterone of <70 ng/dl was taken as normal. Liver function test, and lipid profile (LP) was done in PCOS patients to diagnose dyslipidemia, normal values taken were total cholesterol (TC) <200 mg/dl, high density lipoprotein (HDL) 40-60 mg/dl, low density lipoprotein (LDL) <130mg/dl, triglycerides (TG) <150 mg/dl and very lowdensity lipoprotein (VLDL) <30 mg/dl. All aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were reviewed. The values of normal AST was 10-28 U/l and the value of normal ALT was 0-31 U/l.

5.2. Ultrasound Diagnosis of NAFLD

All patients meeting the inclusion criteria were submitted to real-time ultrasonographic studies of the liver with 3.5 MHz convex transducer and images obtained were sagittal view of the right lobe of the liver and right kidney, transverse view of the left lateral segment of the liver and spleen, transverse view of the liver and pancreas, and any focal areas of altered echotexture- (Samsung HS 60 ultrasound machine) was used for the scan. Curved array transducers with wide-band frequency 4-2 or 5-2 MHz were used for routine abdominal scanning. In obese women or difficult sound beam penetration, a wide-band frequency 4-1, 42, or 3-2 MHz phase array transducer was used for the scanning. The scans were performed in sagittal, coronal, and oblique sub costal planes in supine and left posterior decubitus positions.For the screening for hepatic steatosis, hepatic parenchymal echo texture was evaluated and compared with the echo texture of the spleen/renal cortex. When iso-echogenic, the liver parenchyma is considered normal, that is, without evidence of steatosis. The presence of hyperechoic hepatic parenchyma (bright liver) is considered a characteristic of hepatic steatosis. Absence of hepatic steatosis (HS): equal echogenicity of hepatic parenchyma to that of the renal cortex with clear visualization of the intrahepatic vessels and diaphragm. Transabdominal pelvic ultrasound was performed for assessing both the ovaries for polycystic ovarian morphology. The examination was performed by radiologist who was unaware of the subject's medical histories and laboratory findings with an HS 60, Samsung Medison. A transabdominal pelvic ultrasound was performed. Polycystic ovarian morphology was defined as the presence of 12 or more follicles measuring 2–9 mm in diameter and/or ovarian volume of 10 ml of at least one ovary. The findings were noted.

6. Liver Elastography

6.1. Procedure Followed During Shear Wave Elastography

The patient was positioned in supine decubitus position with the hand above the head as this increases the intercostal space. Patient was asked to do a shallow breath hold, and avoid deep inspiration or deep expiration. To optimize the results, the measurement was taken during a brief breath hold (of a few seconds), because deep breathing and the Valsalva maneuver both change the hepatic venous pressure and can thus alter the assessment of stiffness. The target area was focused roughly two centimetres below the liver capsule, because the correct spot of the region of interest is roughly around four to five centimetres from the transducer surface. The area analyzed was a 10 × 5 mm rectangle that can be freely moved in the two-dimensional (B-mode) image to a maximum depth of 80 mm below the skin surface, which allows a more appropriate measurement in obese patients and in patients with ascites. The rectangular box was perpendicular to the transducer as well as the capsule of the liver. Measurements were performed in segments 5 and 8 of the right lobe of the liver, in the intercostal spaces. It was ensured that area of interest was not within vessels or large biliary radicals. As stated by guidelines 10 readings were taken at the same spot for adequate interpretation. The readings were obtained in kilopascals.

6.2. Result Interpretation

The readings obtained in kilopascals were interpreted according to the Shear wave liver elastography scoring: 2 to 7 kPa- Fibrosis score F0 to F1: no scarring/mild liver scarring.

7.5 to 10 kPa - Fibrosis score F2: moderate.

10-14 kPa -Fibrosis score F3: severe.

14 kPa or higher- Fibrosis score F4: advanced.

6.3. Statistical Analysis

All the data were analysed using SPSS package (Stata, version 26.0 SPSS INC, Chicago, IL, USA) for windows. The data were presented as descriptive statistics for continuous variables and percentage

for categorical variables and was subjected Chi-square test & 't' test other values were represented in number, proportions (%) and mean ± SD.

7. Results

This cross-sectional observational study was conducted in the department of Obstetrics and Gynaecology, AIIMS Patna after obtaining approval from the Institutes Ethics Committee. 86 PCOS patients were recruited in the study from the outpatient department after taking informed consent. All findings and investigations were recorded in the master chart. The results were evaluated using the SPSS software version 26. The results are as follows:

Out of 86 PCOS patients, 36 patients had NAFLD (41.87%) and 50 patients had no NAFLD (58.13%). Table 1 shows demographic profile, clinical profile and laboratory parameters of the PCOS patients with and without NAFLD. Table 2 shows the logistic regression analysis of the variables.From the binary logistic regression, it is observed that Overweight patients have 27 times more risk of developing NAFLD condition (OR 27.429, 95% CI 5.69-132.27), whereas Obese patients have 36 times risk (OR 36.00, 95% CI 5.65-229.29) of developing NAFLD condition compared to normal people. From this analysis we can state that higher BMI is associated with developing of NAFLD. Also, it is seen that the patients whose waist circumference was > 88 cm have 10 times more risk of developing NAFLD which reached a statistical significance (OR 10.560, p value-0.004) proving its association with NAFLD. Hip circumference of >103 cm has 3 times risk of developing NAFLD (OR 3, 95% CI 0.9109.889). From this analysis we can state that higher hip circumference is associated with developing of NAFLD. Waist/Hip ratio of ≥0.8 was also noted to have 89 times more risk of developing NAFLD (OR 89, 95% CI 17.767-448.341) hence proving its strong association. Higher testosterone levels >70 ng /dl were associated with 1.35 times more risk of developing NAFLD (OR 1.352, 95% CI 0.552-3.311). Higher OGTT values >140 mg/dl were associated with 1.292 times more risk of developing NAFLD. Similarly, ALT > 28 iu/l and AST > 31 iu/l were associated with 2.033 and 1.302 times risk of developing NAFLD. Total cholesterol >200 mg/dl was associated with 3.667 times more risk of developing NAFLD (OR 3.667 95% CI 1.22-11.004). Also, triglycerides level > 150 mg/dl is associated with 2.571 times risk of developing NAFLD (OR-2.571 CI 95 % 1.047-6.317). HDL values were found to be lower in NAFLD patients and were found to confer more risk of developing NAFLD. LDL values more than 130 mg/dl were found to have 2.833 times more risk of developing NAFLD (OR 2.833, CI 95 %, 1.127-7.121). VLDL values more than 30 mg/dl has 2.547 times more risk of developing NAFLD (OR 2.547 ,CI 95% , 1.025- 6.324).Clinical feature of hirsutism has 2.532 times more risk of developing NAFLD. (OR 2.532, 95% CI, 0.988-6.494).

8. Discussion

In our study, the prevalence of hepatic steatosis (NAFLD) in patients with PCOS was studied. Additionally, factors associated with the presence of NAFLD in patients with diagnosed PCOS were also investigated. We observed a high prevalence of NAFLD in patients with PCOS (41.87%). In the previous studies conducted, the observed prevalence of NAFLD in patients with PCOS varied significantly. A higher prevalence was observed in some studies while a lower prevalence was observed in other studies. The prevalence of NAFLD in our study was 41.87 %, a rate similar to the one observed in the study conducted by C Cerda et al [4]. This discrepancy may be due to different clinical and metabolic profiles of the studied populations, considering the impact of ethnic variability on the characteristics of PCOS and prevalence of NAFLD. Future studies may help to better elucidate the prevalence of NAFLD in patients with PCOS in different populations. The higher prevalence of hepatic steatosis observed in predominantly overweight patients with PCOS corroborates the hypothesis of the association between NAFLD and PCOS. Additionally, from the logistic regression the diagnosis of PCOS was associated with an increased risk of NAFLD. The high prevalence of obesity in our patients with PCOS compared with that in most studies performed in other countries is a warning of the alarming growth of obesity. Additionally, PCOS women were divided into sub groups based on the presence of a normal hepatic ultrasound or hepatic steatosis on ultrasound (NAFLD). PCOS patients in the two groups had similar age. The mean age of the patients was 27.70±5.99 and majority of the patients belonged to the age group 21-25 years (38.37%). The greater weight of NAFLD patients, compared to non-NAFLD patients, approached statistical significance. A strong correlation was found between BMI and the number of patients affected with NAFLD. Also, NAFLD patients had significantly more waist circumference and hip circumference than NON- NAFLD group. The two groups of PCOS patients had similar luteinizing hormone (LH), follicle stimulating hormone (FSH). It was also observed that patients with NAFLD had higher serum testosterone levels as compared to patients without NAFLD although

Table 1: Demographic profile, clinical profile and laboratory parameters of the PCOS patients with and without NAFLD.

Variable	Overall (n=86)	With NAFLD (N=36)	Without NAFLD (n=50)	P value
Mean age in years	23.71±5.99	23.86±5.51	23.6±6.37	0.843
Mean weight in kg	63.27±4.92	64.47±5.41	62.4±4.39	0.054
Mean height in cm	156.21±5.73	154.83±6.24	157.2±5.17	0.058
Mean BMI	26.2±2.79	27.42±3.24	25.33±2.01	<0.001
Waist hip ratio	0.82±0.05	0.86±0.04	0.79±0.03	<0.001
Menstrual irregularity (%)	59.3	66.7	54	0.238
Infertility (%)	25.6	36.1	18	0.058
Hirsutism (%)	30.2	41.7	22	0.05
Acanthosis nigricans (%)	19.8	27.8	14	0.113
Acne (%)	23.3	30.6	18	
Mean LH in mIU/mL	10.77±2.89	10.96±3.45	10.63±2.43	0.603
Mean FSH in mIU/mL	7.88±2.19	7.92±2.64	7.84±1.83	0.892
Mean serum Testosterone in ng/dl	59.26±15.74	62.36±14.64	57.02±16.26	0.121
Mean OGTT (mg/dl)	138.88±8.4	140.5±11.07	137.72±5.63	0.131
Mean ALT (U/L)	26.44±13.15	29.28±12.76	24.40±13.12	0.09
Mean AST (U/L)	22.14±13.66	25.19±13.87	19.94±13.22	0.078
Mean Total cholesterol (mg/dl)	176.22±36.04	185.31±43.28	169.68±28.49	0.047
Mean triglycerides (mg/dl)	142.94±26	149.64±26.94	138.12±24.44	0.042
Mean HDL (mg/dl)	52.89±8.94	50.45±8.48	54.64±8.93	0.031
Mean LDL (mg/dl)	99.9±32.73	108.11±36.53	94±28.64	0.048
Mean VLDL (mg/dl)	28.16±5.53	29.55±5.36	27.15±5.49	0.047

Table 2: Logistic regression analysis of the variables in our study.

			OR	95% C.I.		p value
	Without NAFLD	With NAFLD		Lower	Upper	
BMI						
<18.5 (Underweight)	0(0)	0(0)				
18.5-24.9 (Normal)	32(64)	2(5.6)	Ref			
25-29.9 (Overweight)	14(28)	24(66.7)	27.429	5.688	132.273	<0.001
30-34.9 (Obese)	4(8)	9(25)	36.000	5.652	229.292	<0.001
>=35 (Morbid Obese)	0(0)	1(2.8)				
Waist circumference(cm)						
<=88 cm.	48(96)	25(69.4)	Ref			
>88 cm.	2(4)	11(30.6)	10.560	2.170	51.386	.004
Hip circumference (cm)						
<=103	45(90)	27(75)	Ref			
>103	5(10)	9(25)	3.000	.910	9.889	.071
Waist/Hip Ratio						
<0.80	42(84)	2(5.6)	Ref			
>=0.80	8(16)	34(94.4)	89.250	17.767	448.341	<0.001
			OR	95% C.I.		p value
	Without NAFLD	With NAFLD		Lower	Upper	
LH						
1.9-12.5	38(76)	22(61.1)	Ref			
<1.9 &>12.5	12(24)	14(38.9)	2.015	.793	5.122	.141
FSH						
2.5-10.2	47(94)	30(83.3)	Ref			
<2.5 &>10.2	3(6)	6(16.7)	3.133	.728	13.487	.125
Serum testosterone (ng/dl)						
<70	34(68)	22(61.1)	Ref			
>=70	16(32)	14(38.9)	1.352	.552	3.311	.509
OGTT (mg/dl)						
<140 (Normal)	24(48)	15(41.7)	Ref			
140-199 (Impaired)	26(52)	21(58.3)	1.292	.545	3.067	.561
ALT(U/L)						
10-28 u/l	37(74)	21(58.3)	Ref			
<10 &>28	13(26)	15(41.7)	2.033	.814	5.079	.129
AST (U/L)						
0-31	41(82)	28(77.8)	Ref			
>31	9(18)	8(22.2)	1.302	.448	3.782	.628
Total Cholesterol (mg/dl)						
<200 mg/dl	44(88)	24(66.7)	Ref			
>=200 mg/dl	6(12)	12(33.3)	3.667	1.222	11.004	.020
Triglycerides (mg/dl)						
<150 mg/dl	36(72)	18(50)	Ref			
>=150 mg/dl	14(28)	18(50)	2.571	1.047	6.317	.039
HDL (mg/dl)						
40-60 mg/dl	35(70)	32(88.9)	Ref			
<40 &>60 mg/dl	15(30)	4(11.1)	.292	.088	.971	.045
LDL (mg/dl)						
<129 mg/dl	38(76)	19(52.8)	Ref			
>=130 mg/dl	12(24)	17(47.2)	2.833	1.127	7.121	.027
VLDL (mg/dl)						
2-30 mg/dl	37(74)	19(52.8)	Ref			
<2 &>30 mg/dl	13(26)	17(47.2)	2.547	1.025	6.324	.044

it did not reach statistical significance. The results also demonstrated that 34.9% of women had elevated serum testosterone values. Similarly, OGTT values were higher in NAFLD group although it did not reach statistical significance. The mean ALT and AST values in the NAFLD group were higher which approached statistical significance.

Patients with NAFLD had higher serum cholesterol, trigylcerides, and serum LDL and VLDL levels than patients without NAFLD. Elevated cholesterol levels were observed in 12 patients with NAFLD and in 6 patients without NAFLD. Elevated LDL levels were observed in 17 NAFLD patients and in 12 patients without NAFLD. Furthermore,

patients with NAFLD had a trend toward a lower HDL than patients without NAFLD. Metabolic syndrome has been characterized by the presence of at least three factors out of five, these include: abdominal obesity, elevated triglycerides, reduced HDL level, hypertension, and impaired fasting glucose levels. These metabolic abnormalities were found to be more in patients with NAFLD.

NAFLD in the general population is frequently associated with obesity, increased BMI, type II diabetes mellitus, dyslipidemia, and metabolic syndrome. Almost all the patients we evaluated had one risk factor, an elevated BMI. The association observed between hepatic steatosis and central adiposity, triglyceride levels, and prevalence of Metabolic Syndrome (MS) corroborates previous studies. According to the knowledge that the components of MS are risk factors for NAFLD and development of advanced liver disease, the investigation of metabolic abnormalities in patients with PCOS is of great importance in clinical practice. The association of Insulin Resistance (IR) demonstrated by deranged OGTT with steatosis confirms the importance of IR in the pathophysiology of both conditions.IR in PCOs patients seems to be multifactorial and might reflect an influence of genetics, obesity, diet, and sedentary lifestyle. Most participants in our study had the PCOS phenotype A or B, which includes clinical or biochemical hyperandrogenism. As the present study was conducted in a reference center, the predominance of classic hyperandrogenic phenotypes may have been related to a selection bias, thus not representing the real distribution in the general population. Hirsutism was present in 23% of the patients with PCOS. Androgenic features like acne was present in 23.25% of the patients, acanthosis nigricans in 19.77 % of the patients, fertility problems in 25.58 % of the patients and Irregularity of menstrual cycle was present in 59.30%.However, the quantification of hirsutism using the Ferriman-Gallwey score is affected by subjectivity, interobserver variability, and ethnic differences. Concerning the biochemical evaluation of hyperandrogenism, serum SHBG measurements were not available and were limited to total testosterone, which is less sensitive than the free androgen index to detect subtle hyperandrogenemia.Although several studies have shown a high prevalence of steatosis in patients with PCOS, the detection of liver disease may still be underestimated. Despite having an acceptable level of sensitivity to detect liver fat, ultrasonography has some limitations, including a lower accuracy in the presence of obesity. Considering that 60% of the patients with PCOS in our study were having BMI >25 it is possible that the prevalence of liver steatosis in our cohort may have been even higher.

In NAFLD, fibrosis is the characteristic associated with the highest mortality risk; therefore, its early detection has great importance. In NAFLD staging in the present study, one patient with PCOS presented evidence of advanced NAFLD score. The natural history of NAFLD in patients with PCOS is poorly understood. As in this study, other recent studies have used noninvasive methods like TE and serum biomarkers such as the FIB-4 index and NALFD score to estimate the liver disease stage more accurately in patients with PCOS. Despite the evidence of a higher prevalence of NAFLD in women with PCOS and its potential for progressive liver disease due to the concomitance of MS factors, the association of NAFLD in patients with PCOS is not widely known by physicians taking care of these patients. In addition, the recommendations to investigate NAFLD in women with PCOS are controversial and the impact of NAFLD in these patients is most likely underestimated. A more precise definition of which factors are implicated in the pathophysiology of NAFLD in PCOS and knowledge of the natural history of liver disease in these patients may allow, in the future, a better selection of risk groups with more precise interventions.The limitations of this study include the facts that alcohol consumption (considered as an exclusion criterion) was self-reported by the patients. Since this prevalence study recruited patients seeking medical evaluation due to symptoms related to PCOS, it is likely that the selected patients had more clinical features than the general population of patients with PCOS. Other limitations are inherent to a cross-sectional study, which fails to clarify whether the clinical conditions associated with the presence of NAFLD in concomitance with PCOS are etiological factors. Prospective cohort studies are required to establish the temporal sequence of events and elucidate the possible cause and effect relationship between PCOS and NAFLD. Also the number of patients in this study was small and they were treated at a single hospital center. This cohort may not be representative of all PCOS patients. Our cohort may have been too small to detect some risk factors or associations with statistical significance. The use of different PCOS diagnostic criteria in the literature affects the ability to compare reported studies. Collaborative national or international studies are needed to define health care risks of PCOS patients, using different PCOS definitions in a better way.

9. Conclusion

Non-alcoholic liver fatty liver disease (NAFLD) is emerging as a common chronic liver disease across the world comprising a spectrum of liver damage from fatty liver infiltration to end-stage liver disease, in patients without significant alcohol consumption. The prevalence of NAFLD has been reported to be more in patients with polycystic ovary syndrome (PCOS). Obesity, in particular central adiposity and insulin resistance are considered as the main factors related to NAFLD in PCOS. There are also data which support that androgen excess, which is also a feature of polycystic ovary syndrome and is interrelated to insulin resistance, may be an additional contributing factor to the development of NAFLD. Although the natural history of NAFLD remains unclear and hepatic steatosis seems to be a relatively benign condition in most patients, limited data imply that advanced stage of liver disease is possibly more frequent in obese polycystic ovary syndrome patients with NAFLD.PCOS patients, particularly obese patients with features of the metabolic syndrome, should be submitted to screening for NAFLD comprising assessment of liver function tests and lipid profile and of hepatic steatosis by abdominal ultrasound. Lifestyle modifications including diet, weight loss and exercise are the most appropriate initial therapeutic interventions for PCOS patients with NAFLD. When pharmacologic therapy is considered, metformin may be used, although currently there is no medical therapy of proven benefit for NAFLD. Long-term follow up studies are needed to clarify clinical implications and guide appropriate diagnostic evaluation, follow-up protocol and optimal treatment for PCOS patients with NAFLD. In conclusion, the present study evaluating the association between NAFLD and PCOS in a tertiary care center demonstrated a high prevalence of steatosis in patients with PCOS. NAFLD is commonly found in women with PCOS, and may be an early sign of metabolic syndrome. Patients with PCOS should be screened for NAFLD as early interventions in patients with can be an important measure to reduce the risk of progression to advanced liver disease..

References

1. Irwing F stein, Michael L leventhal. Ammenorhoea associated with bilateral polycystic ovaries. ACOG. 1935; 29: 1-5
2. Gambarin Gelwan M Kinkabwala, SV. Prevalance of Non alcoholic fatty liver disease in women with PCOS. World J gastroenterol. 2015.
3. Gambarin-Gelwan M, Kinkhabwala SV, Schiano TD, Bodian C, Yeh HC. Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. Clin Gastroenterol Hepatol. 2007; 5(4): 496-501.
4. Cerda C, Pérez-Ayuso RM, Riquelme A, Soza A, Villaseca P. Nonalcoholic fatty liver disease in women with polycystic ovary syndrome. J Hepatol. 2007; 47(3):412-7.
5. Wu J, Yao XY, Shi RX, Liu SF, Wang XY. A potential link between polycystic ovary syndrome and non-alcoholic fatty liver disease: an update meta-analysis. Reprod Health. 2018; 15(1): 77.
6. Brzozowska MM, Ostapowicz G, Weltman MD. An association between non-alcoholic fatty liver disease and polycystic ovarian syndrome.JGastroenterolHepatol. 2009; 24(2): 243-7.
7. Zhag J, Hu J, Zhang C, Jiao Y, Kong X, Wang W. Analyses of risk factors for polycystic ovary syndrome complicated with non-alcoholic fatty liver disease. Exp Ther Med. 2018; 15(5):4259-64.
8. Vassilatou E, Vassiliadi DA, Salambasis K, Lazaridou H,

Koutsomitopoulos N, Kelekis N. Increased prevalence of polycystic ovary syndrome in premenopausal women with nonalcoholic fatty liver disease. Eur J Endocrinol. 2015;173(6):739-47.

9. Hossain N, Stepanova M, Afendy A, Nader F, Younossi Y, Rafiq N, Goodman Z, Younossi ZM. Non-alcoholic steatohepatitis (NASH) in patients with polycystic ovarian syndrome (PCOS). Scand J Gastroenterol. 2011; 46(4): 479-84.
10. Kelley CE, Brown AJ, Diehl AM, Setji TL. Review of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. World J Gastroenterol. 2014; 20(39): 14172-84.
11. Mehrabian F, Jahanmardi R. Nonalcoholic fatty liver disease in a sample of Iranian women with polycystic ovary syndrome. Int J Prev Med. 2017; 8: 7.
12. Al-Jaroudi D, Jamaat S, Kaddour O, Wani T, Al-Badr. Non-Alcoholic Fatty Liver Disease in Infertile Women with Polycystic Ovarian Syndrome: A Prospective Series. Int J Womens Health Wellness. 2017; 3: 047.
13. Mariana DR. Prevalence of non-alcoholic fatty liver disease in women with polycystic ovary syndrome and its correlation with metabolic syndrome. ArqGastroenterol. 2015; 52(2): 1-5.
14. Tracy L. Setji, Nicole D. Holland, Linda L. Sanders, Kathy C. Pereira, Anna Mae Diehl, Ann J. Brown. Nonalcoholic Steatohepatitis and Nonalcoholic Fatty Liver Disease in Young Women with Polycystic Ovary Syndrome. J ClinEndocrinolMetabol. 2006; 91 (5): 1741-7.
15. Thomas M Barber, Mark I McCarthy, Stephen Franks,John AH Wass. Diabetes Research Laboratories, Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford University, Oxford, UK 2 Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford University, Oxford, UK 3 Institute of Reproductive and Developmental Biology, Imperial College, London, UK. 2008.
16. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM. NASH and insulin resistance: In-sulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology. 2002; 35: 373-379.
17. Abdelmalek MF, Diehl AM. Nonalcoholic fatty liver dis- ease as a complication of insulin resistance. Med Clin North Am. 2007; 91: 1125-1149.
18. Angelico F, Burattin M, Alessandri C, Del Ben M, Lirussi F. Drugs improving insulin resistance for non-alcoholic fatty liver disease and/or non-alcoholic steatohepatitis. Cochrane Database Syst Rev. 2007; (1): CD005166.
19. Yilmaz Y. Review article: is non-alcoholic fatty liver disease a spectrum, or are steatosis and non-alcoholic steatohepatitis distinct conditions? Aliment Pharmacol Ther. 2012; 36: 815-823.
20. Wicks IP, Cooke BE, Allen R, Bertouch JV. Aortic incom- petence associated with severe rheumatoid arthritis. Med J Aust. 1987; 146: 317-320.
21. Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Li- cholai T. Evidence for distinctive and intrinsic defects in in- sulin action in polycystic ovary syndrome. Diabetes. 1992; 41: 1257-1266.