

Prevalence of non alcoholic fatty liver and Non alcoholic Steatohepatitis in Peshawar Cantonment, Khyber Pakhtunkhwa, Pakistan

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Abstract: Non-alcoholic fatty liver disease prevalence has not been well established. The aims of this study was to define prospectively non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD) prevalence in hospitalized and ambulatory patients 20-65 years old during June 2013 to June 2014 were selected from Combined Military Hospital Peshawar Cantonment area. A base line questionnaire and right upper quadrant ultrasound was completed by all patients. On identifications of fatty liver among the selected cases further lab test data and liver biopsy reports were obtained. Mean BMI of female was 29.9 ± 5.65 while prevalence of hypertension and diabetes was 49.8% and 16.6% respectively. Among all patients 62% were Punjabis, 23% were Pathans while 12% were Sindhis. Overall NAFLD prevalence was 47% while NASH was confirmed in 20 patients (12.3% of total and 30% of ultrasound positive patients). Pathans had the highest prevalence of NAFLD (58.5%) as compared to Punjabis (44.5%) and Sindhis (35.3%). Pathans also had a higher prevalence of NASH compared with Punjabis (19.5% VS 10%: $P=0.03$). In general, NAFLD patients were more prevalent among male (59%), Diabetic ($P<0.00005$), hypertensive ($P<0.00005$) and older ($P=0.005$). They consumed more fast food ($P=.049$) had a higher BMI ($P<0.0005$) and had little or no exercise as compared to their normal or non NAFLD counter parts ($P=0.02$). NAFLD was found in 75% and NASH in 22.5% among the 26 diabetic patients. ALT, AST, BMI, insulin, quantitative insulin sensitivity checks index and cytokeratin – 18 correlated with NASH. It was concluded that NAFLD and NASH prevalence is higher than estimated previously, Pathans and Patients with diabetes are at high risk.

Keywords: Prevalence, fatty liver disease, NAFLD, NASH, ultrasound, Liver biopsy.

INTRODUCTION

Worldwide prevalence of obesity and its associated liver injuries called NAFLD (non-alcoholic fatty liver disease) is on increase. It is estimated that around 35% of adult population in developed countries is suffering from obesity and its related conditions including NAFLD and NASH (Adams *et al.*, 2005). Even in children the condition is on increase. NAFLD is to be suspected in those cases showing simple clinical signs and lab profile consistent with insulin resistance. A significant number of cases with NAFLD, often considered a benign condition, will lead to more severe liver disease including non-alcoholic steatohepatitis (NASH) with or without Fibrosis, liver cirrhosis and sometime liver cancer (Ruopeng A 2014). NAFLD is also commonly associated with metabolic syndrome, high risk of type-2 diabetes and manageable features of insulin resistance such as dysglycemia and dyslipidemia (McCullough 2006). Histopathological assessment of liver tissue is the most proven way of differentiating simple steatosis from NASH, a condition more likely to progress to hepatic cirrhosis.

NAFLD is now considered the leading cause of abnormal liver enzymes that is associated with higher risk of developing coronary artery disease and diabetes (Rubinstein *et al.*, 2008). Worldwide NAFLD prevalence has been estimated to be ranging from 3% to 45%. Wide variations in prevalence depend upon study population and the diagnostic tools used e.g. liver biopsy, imaging and liver enzyme etc (Lazo M, Clark JM 2008). In a study utilizing proton nuclear magnetic resonance spectroscopy NAFLD prevalence was estimated 31% (Phunchai *et al.*, 2012, Browning JD *et al.* 2004). In this study mean age cohort was 54.4 ± 7.30 year having age range (20-60 years) depending on ethnicity and sex, with total obesity prevalence of (43%). Hispanics are on the top with 45% prevalence ratio, followed by Caucasian having 33% occurrence and 24% in African Americans. The prevalence of non-alcoholic steatohepatitis (NASH) is very difficult to be estimated because for diagnosis liver biopsy is required. The prevalence of non-alcoholic steatohepatitis (NASH) in general population globally has not been well defined. In early 80's from an autopsy study the prevalence of NASH was found to be 2.7% among smart and lean individuals, in obese individual this raised to 18.5% (Yoshihiro I 2014). Recently three different studies while evaluating liver transplantation donor NASH prevalence estimated to be 1.1% to 14%. There

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was no report of alcohol consumption in these cases (Halon *et al.*, 2006). The prevalence of NASH and NAFLD varies with population uniqueness and variation. In obese patients undergoing bariatric surgeries the prevalence of NAFLD and NASH has been found up to 91% and 37% respectively (Lazo, 2008; Vander *et al.*, 2014), while studying diabetics patients. NAFLD prevalence was found to be 40% to 69% without data for the NASH prevalence.

Aim of work

The aim of this study was to prospectively define the prevalence of NAFLD and NASH in selected population using various diagnostic tools at the department of Medicine Combined Military Hospital Peshawar Cantonment.

MATERIALS AND METHODS

This prospective study was conducted in the department of Medicine at the Combined Military Hospital Peshawar Cantonment, a tertiary care hospital of Khyber Pakhtunkhwa, Pakistan. Only eligible cases including Army personnel, Officers and their families, parents and private patients who visited department of Medicine for various medical problems in age group of 20 to 65 years were enrolled for the study. It includes patients of both gender. Diagnosis was made on the basis of ultrasound and liver biopsy along with clinical findings. All specialists and medical officers (MO's) were informed about study scope and patients referral for enrollment. Approximately 400 patients were offered enrollment during 12 months period from June 2013 to June 2014 after permission from hospital ethical committee and written patient consent. A base line questionnaire was provided. The questionnaire includes information about the patients' dietary habits i.e. frequency of fast food restaurants visits and soft drinks / beverages intake, medical history, known history of chronic liver disease and current drug profile. Following categories of patients were excluded; having a history of chronic liver disease or on medications that are associated with fatty liver disease steroids, tamoxifen etc.

Sampling methods of NAFLD and NASH

Ultrasound right upper quadrant and percutaneous liver biopsy were used as diagnostic tools for NAFLD and NASH.

U/s right upper quadrant

Ultrasound right upper quadrant was performed by radiology department at Combined Military Hospital Peshawar Cantonment. Ultrasonographic examinations were performed by qualified radiologist having 10-15 years of experience. For right upper quadrant ultrasound examination technical parameters were adjusted for each patient using the standard protocols. The liver was labeled

as fatty liver when the liver had areas of significantly increased echogenicity as compared to the renal parenchyma.

Liver biopsy

All cases among the studied patients having a positive US report i.e. suggesting hepatic steatosis were considered for liver biopsy. Venous blood was drawn in the morning after an overnight fast and following fasting blood tests were carried out before liver biopsy: Complete blood counts, coagulation studies, lipid profile, liver function tests, hemoglobin A1C, blood glucose, serum adipokine and cytokine analysis. An expert hepatologist himself did all the biopsies using 18 G trucut biopsy needle. All the biopsy were examined and reported by expert histopathologists utilizing the Brunt system of grading and staging of steatohepatitis.

STATISTICAL ANALYSIS

Potential difference between proportions and rates of occurrence were assessed with fisher exact test or χ^2 as appropriate and descriptive statistics were calculated for all age groups. The data was analyzed using SPSS version 11.0 computer software. Differences of the average value between groups for continuous variables were evaluated with Mann Whitney test. A logistic regression model was constructed and parameters selected by maximum like to assess the independent predictive effect of a covariate for a nominal response (development of NASH). On a spearman rank correlation analysis only the factors that were significant ($P < 0.05$) were covered in to the multivariate model for the determinations of independent prognostic effect of these variables for development of NASH. For the best predictive model receiver operating characteristic curve was created. The probability of NASH presence was calculated by utilizing the combination of minimum maximum and mean values from the logistic regression model of independent predictors.

RESULTS

A total of 200 patients were enrolled from June 2013 to June 2014, 30 patients either did not underwent ultrasound examination or did not keep their ultrasound record, 06 patients were excluded on having earlier fatty liver disease, 164 Patients underwent right upper quadrant ultrasound. Most of the patients were middle aged (53.5 ± 7.30 year, ranges 20-65 years) and 51% were female. The mean BMI calculated as Kg/M^2 was 29.9 ± 5.65 with 45.5% of the cases meeting obesity criteria ($\text{BMI} > 30$). Hypertension and diabetes mellitus were identified in 49.8% and 16.6% of patients respectively.

Table 1: Clinical demographic data

N=164	Negatives U/S (Non NAFLD) (n=86)	Positive U/S NAFLD include NASH) (n=78)	P Value
Study Cohort %	54% (n=86)	46% (n=78)	NA
Pathans (n=36) % *L= Pashtu	41.5% (n=36)	58.5% (n=36)	NA
Punjabies (n=103) % *L= Punjabi	55.5% (n=103)	44.5% (n=103)	NA
Sindhies (n=18) % *L= Sindhi	64.8% (n=18)	35.2% (n=18)	NA
Others (n=7) % *L= Hindko	64.4% (n=7)	35.6% (n=7)	NA
Male %	41%	59%	0.002*
Age, γ , mean(SD)	53.7% (7.85)	55.8% (6.50)	0.005*
BMI a, Mean (SD)	27.5% (4.95)	32.5% (5.35)	< 0.000
BMI $\geq$ 30 (obese)%	26.7%	67.6%	<0.000
Diabetes %	10.1%	26.5%	<0.000
Hypertension %	33.8%	68.5%	<0.000
Fast Food ($\geq$ 2 per week)	60.7%	70.8%	0.049*
Non diet Beverages ($\geq$ 2 per week)	39.7%	48.5%	0.12*
Exercise ($\geq$ 60 min / week)	68.8%	56.5%	0.02*

Table 2: Clinical Data between NASH and without NASH patients

N=164		Without NASH (N=46)	NASH (N=20)	P Value
Complete Study prevalence cohort %		28.1	12.3	NA
Pathans (n=18) % *L= Pashtu		59	41	NA
Punjabies (n=39) % *L= Punjabi		37.6	26.4	NA
Sindhies (n=06) % *L= Sindhi		61.6	38.4	NA
Others (n=3) % *L= Hindko		79.8	19.2	NA
Male%		59	64	0.55*
Age,γ,mean (SD)		55.2(6.76)	54.18(6.16)	0.40*
BMI a Mean (SD)		31.7(5.35)	34.5(5.42)	0.02*
BMI >30(obese)%		64.5	80.5	0.08*
Diabetes		23.9	25.2	0.87*
Hypertension%		63	77.6	0.11*
Insulin- Sensitivity check Index quantitative mean (SD)		0.34(0.04)	0.31(0.03)	<0.00
Glucose, mg/dl, mean (SD)		103.5(30.02)	109.6(26.9)	0.18
Insulin, iu/ml, mean(SD)		15 (8.5)	23.5(14)	<.0005
Hb A1c, mean (SD)		06.14(0.92)	06 (0.73)	0.83*
ALT, U/L, mean (SD)		36.3(15.74)	51(19.56)	<0.0005
AST, U/L, mean (SD)		25.7(7.45)	36.4(13.06)	<.0005
Alkaline phosphates mg/ml mean (SD)		82.8(22.32)	88.3(29.92)	0.26*
LDL mg/dl, mean (SD)		110.7(39.25)	111.4(30.07)	0.94*
HDL mg/μl, mean (SD)		49.3(15.66)	44.4(0.02)	0.04*
Total cholesterol mg/ml mean (SD)		180.7(44.50)	187(36.70)	0.35*
Adiponectin mg/ml, mean (SD)		11,030(13,080)	7816(4812)	0.03*
Tumor necrosis factor, pg/ml mean (SD)		5.59(2.05)	12.04(36.03)	0.14*
High sensitively c-reactive protein, mg/ml, mean (SD)		5356(5538)	7352(6398)	0.05*
Cytokaratin-18 μg/l, mean (SD)		210.4(119)	307.2(233.2)	0.03*
Free fatty acid, mEq/l, mean(SD)		0.63(0.27)	062(0.22)	0.89*
Steatoosis %				
	<05	19	0.0	NA
	05-33	59.7	37.7	NA
	33-65	21.4	51	NA
Beverages Non diet >02 per week) %		43	55.5	0.21*
Fast food >2 per week) %		67.5	80.2	0.15*
Exercise (> 60 min per week) %		56.45	57.7	0.90*

Key: *L stand for Language spoken

Prevalence of NAFLD

Seventy eight patients out of 164 had evidence of NAFLD upon ultrasound of right upper quadrant and were referred for percutaneous liver biopsy, ten patients refused percutaneous liver biopsy and three patients had no NAFLD evidence upon biopsy. The majority of this study NAFLD cases were male (59%). NAFLD cases were heavier and significantly older with a much higher ratio of prevalence of diabetes and hypertension NAFLD cases also visited more fast food restaurant and their eating habits were irrational also they exercised very less as compared to other normal healthy individuals.

Prevalence of NASH

The NASH prevalence was found 12.3% (20 patients) among the entire cohort. The majority (65%) of NASH patients were male with more insulin resistance and a significantly higher BMI than patients with simple steatosis on liver biopsy (table 2). The mean AST (aspartate amino transferase) (36.5 ± 13.3 vs 25.8 ± 7.45 and mean ALT (alanine aminotransferase) (50.9 ± 19.8 vs 36.4 ± 15.9) were higher in the NASH cohort significantly ($P < 0.0005$). The means HDL (high density lipoprotein) levels were significantly low ($P = 0.04$) although there was no significant difference in LDL cholesterol level (low density lipoprotein), Cytokeratin 18 and C-Reactive protein levels were ($P = 0.03$) and ($p = 0.05$) respectively which were significantly higher among the NASH patients while adiponectin ($P = 0.03$) was significantly lower among the NASH cohort. On observation NASH patients and the simple steatosis cases with respect to free fatty acid or tumor necrosis factor levels no difference were noted. A significant correlation with respect to following variable was found for NASH: ALT (0.390; $P < 0.002$), AST (0.315; $P < 0.002$), Insulin (0.412; $P < 0.002$), BMI (0.224; $P = 0.02$), Insulin sensitivity check index quantitative (0.378; $P < 0.002$), and cytokeratin-18 (0.277; $P = 0.003$), insulin and alanin aminotransferase were found predictive of NASH independently.

DISCUSSION

NAFLD the ectopic fat accumulation in the liver with possible progression to NASH and cirrhosis is more common and well linked to obesity and insulin resistance. In this prospective study the most compelling finding is the overall prevalence of NAFLD (46.5%) and NASH (12.3%) within our middle aged population. The Dallas heart study found an overall prevalence for NAFLD 31% by utilizing more sensitive imaging modalities. According to Dallas Heart study, the highest prevalence rate was found in Hispanics followed by Caucasians and African American (Browning JD *et al* 2004). A higher prevalence of NAFLD in this study may be due to very simple and less sensitive tests used for the study and a rising trend of obesity and diabetes in Pakistan like around the world.

Life style mediated weight loss, through intake of reduced calories and physical exercise is generally recommended to reduce liver fat content. The significant difference between patients with fatty liver group and those having no NAFLD on U/S studies is really striking. Cases labeled with fatty liver visited more fast food restaurants and seen less involved in any physical exercise compared to other counterparts without fatty liver disease. Other investigators have also confirmed linkage of fatty liver disease to life style. (Chrystofer *et al.*, 2011) found in their study that the majority of patients did not meet the physical activities recommended guidelines for NAFLD (Brunt *et al.*, 1999). Other investigators have shown that diet higher in simple carbohydrates and less in vitamins especially vitamin C & E and dietary fibers was consumed by NASH patients (Toshimitsu *et al.*, 2007). Data have shown that survival rate and quality of life is lower in NAFLD cases as compared to general population (Adams *et al.*, 2005, Rafiq *et al.*, 2009).

Liver related mortalities are considered the 3rd cause after cardiovascular and malignant disease. NAFLD cases were asymptomatic and were in middle aged population in our study whereas NASH prevalence is 7 to 8 fold higher than previously investigated (Armstrong *et al.*, 2006). Accordingly, to our knowledge at Pakistan Army level this is the first study to prospectively define prevalence NAFLD and NASH. 3% of cases had evidence of advanced NASH i.e. stage 2-4 fibrosis. In patients with NASH long term follow up studies are lacking, but it has been shown that in NASH patients liver related mortalities, are higher as compared to non-NASH fatty liver (Caballeria *et al.*, 2010, Denzer *et al.*, 2010). In 20% cases cirrhosis develops whereas 30% to 40% of cirrhotic patients dies during 10-years period, which is similar to mortality rate with chronic hepatitis C (McCullough 2006).

NASH prevalence varies according to ethnicity; prevalence is highest in Pathans in this region. It has been found that genetics and life style play a great role. Examples include TNF α polymorphism (Bornard *et al.*, 2000). The PNPLA, 3 genes have been linked to steatosis and hepatocyte injury which is due to genetic polymorphism. It may be more common among Pathans like Hispanics (Williams *et al.*, 2011, Ruhl *et al.*, 2015). However, such genetic studies are smaller and require much higher studies for confirmation.

The sex plays a major role in the prevalence of NAFLD. During our study it was found that male patients had significantly higher rate of NAFLD (59% VS 41%; $p = .001$) and also higher rate of NASH (64% VS 35%; $P = .02$). Sex hormone metabolism may be important in pathogenesis of NAFLD these findings are consistent with previous studies (Peterson *et al.*, 2010). These statements are strongly supported by recent study in

pediatrics that found highest rate of fatty liver disease in boys after puberty (51.3%) and the lowest rate of NAFLD in postpubertal girls (12.3%) (Caballeria *et al.*, 2010). Male patients with NAFLD within this study cohort had significantly lower alkaline phosphatase (79.7%+ 22.5 VS 92.5 + 27.5; $p=0.005$), and higher serum ALT (44.2 + 18.2 VS 35.5 + 17.5: $p=0.008$), mean HDL (43.5 + 9.6 VS 54.5 + 17.5: $P<0.00005$), C reactive protein (5.65 + 6341 7315 + 6380; $p= 0.02$), adiponectin (7875 + 4235 VS 11505 + 9370; $p<0.000$). In male and female patients with NAFLD no difference in insulin resistance and obesity were found. Although life style difference between male and female may play a role because non diet beverages were consumed more by male patients on weekly basis (54.5% Vs 35%, $p=0.038$).

The diabetic patient cohort was of special interest because they are at high risk for NAFLD as compared to general population. While going through some previous studies on the subject matter the prevalence of NAFLD is around 60% which is nearly consistent with results found in our study (Denzer *et al.*, 2009, Kelley *et al.*, 2003). In Pakistan NASH prevalence had not been defined prospectively till date according to our knowledge. In this study the prevalence of NASH in diabetic population was found to be 23% which is higher significantly than in non-diabetic patients' prevalence (11%, $p=0.04$) which is quite a matter of worry because of higher risk of mortalities in patients with diabetes (Flegal *et al.*, 2010).

Every research study certainly has some limitations. So limitations of this study are acknowledged because this is not a study based on population but on case ascertainment and selection basis. The interesting fact regarding the prevalence of obesity, diabetes and hypertension in this study is that it is quite similar to that found by various US age based population studies (Williams *et al.*, 2011, Flegal *et al.*, 2010, Ruopeng 2014). No similar studies giving this type of statistics are available in Pakistan till date on subject matter. It is possible that these observation and findings are conservative and NAFLD and NASH prevalence may be higher. In this study ultrasound right upper quadrant was used as a screening technique for detection of fatty liver. Although the specificity and sensitivity of ultrasound is very good for detection of fatty liver, whereas in cases where steatosis is accounting for <33%, the accuracy of the test is likely to be diminished.

CONCLUSION

NAFLD the ectopic fat accumulation in the liver with possible progression to NASH and cirrhosis is clearly linked to obesity and insulin resistance. NAFLD and NASH prevalence are higher than estimated previously among the cohort of middle age patients. This raises serious and significant public health concerns. Although all ethnic groups are affected, insulin sensitivity varies

among patients, Pathans and Patients with diabetes are at high risk for fatty liver disease including both NAFLD and NASH.

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