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Study of Biochemical Parameters as Predictive Factors of Nash

Pandit Vinodh.Bandela[1], Havilah polur[2] , Durga Prasd Kedam[3] , Ramakumar[4] , Hussain Saheb Shaik[5].

[1,2,3 & 4] *Department of Bio Chemistry, Santhiram Medical College, Nandyal, Andhrapradesh.*
[5] *Dept of Anatomy, JJM Medical College, Davangere.*

Abstract

Aim:

The aim of our present study was to assess the clinical markers of metabolic syndrome in Non alcoholic steatohepatitis (NASH) patients.

Methods:

we selected two groups of patients, NASH (n=40) and Cirrhotic (n=40) from Santhiram General Hospital Nandyal, based on Ultrasonography findings. Inclusion criteria was taken into consideration in selecting the. The anthropometric, clinical and biochemical blood investigations included WC, Blood pressure and FBS, Lipid profile, liver function tests, insulin respectively. Homeostatic Metabolic Assessment (HOMA)-index was evaluated. The factors associated with NASH and Cirrhosis was identified. Pearson's Correlation of factors with IR was done. Finally sensitivity, specificity and predictive values were calculated.

Results:

At univariate analysis, of metabolic syndrome parameters were elevated in NASH group. And the parameters were significantly correlated with IR. Aminotransferase levels were found to be in uppar normal range in NASH and progressively increased in cirrhosis (AST 52.8±12 vs. 319±133 and ALT 50.83±10.19 Vs.325±18.19 respectively) .In our study we found 100% positive predictive value for FBS, followed by IR (96.69%), WC (92.86%), and TGL (87.50%) respectively. Our data also shows highest specificity for FBS (100%), followed HOMA IR (96.67%), WC (93.33%) and TGL (86.67%).

Conclusion:

A simple non invasive predictive model that incorporates both clinical and biochemical parameters of metabolic syndrome can identify patients at risk with NASH.

Abbreviations:

NASH- Nonalcoholic steatohepatitis; NAFLD -Nonalcoholic fatty liver disease; HOMA- Homeostatic Metabolic Assessment; WC-Waist Circumference; ALT- Alanine aminotransferase; AST-Aspartate aminotransferase; US-Ultrasonography;PPV- Positive Predictive Value; NPV- Negative Predictive Value.

INTRODUCTION:

Nonalcoholic fatty liver disease (NAFLD) was first introduced by Ludwing and his colleagues at mayo clinic in 1980, defined as more than 5% of the hepatocytes containing fat or more than 5% of the liver weight due to fat[1]. Now it was considered as most common liver disease and involves a spectrum of progressive histopathologic changes. Common risk factors associated with NAFLD includes Obesity, Diabetes, and Hyperlipidemia. Although most of the patients have simple hepatic steatosis and a significant number develop NASH. This may progress to fibrosis, Cirrhosis or end stage liver disease. There is increasing evidence that NAFLD is a common feature in patients with the metabolic syndrome (MS), a constellation of metabolic, cardiovascular, renal and inflammatory abnormalities in which insulin resistance is thought to play a key role in end organ pathogenesis. NAFLD is usually diagnosed after abnormal liver chemistry results are found during routine laboratory testing. No therapy has been proven effective for treating NAFLD/NASH. Currently, for NASH diagnosis liver biopsy is the Gold standard method which is invasive technique, with drawbacks of sampling and interpretation errors hence there is a need for non-invasive strategies to cover the whole spectrum of NAFLD is a priority. It is also necessary for future research to develop an algorithm for Non alcoholic fatty liver disease (NAFLD) patient screening and to identify patients at risk for the progression liver disease.[2]

AIM OF THE STUDY:

The aim of our study is to assess the importance of biochemical parameters and risk factors for early detection of NAFLD

MATERIALS AND METHODS:

Study population

we selected the subjects for the current study based on Ultrasonography findings, those individual with fatty infiltration at gray scale on Ultrasonography were taken as NASH (N=30) and those with significant fibrosis (grade >4) were taken as cirrhosis (N=30). Inclusion criteria for consisted of the absence of significant alcohol abuse <20gr./day (confirmed by at least one family member), no evidence of hepatitis B and C and of drug induced liver disease and no other specific liver diseases were taken into consideration in selecting the patients. All the 60 subjects underwent physical examination, anthropometric measurements and biochemical blood investigations. Metabolic syndrome was defined according Modified ATPIII Criteria The study was approved by the local ethics committee and all individuals provided written informed consent prior to enrolment in the study.

Clinical evaluation

Waist circumference (WC) is measured at the level of umbilicus, > 90cm in male, >80 cm female was considered as obesity**.** Systolic / diastolic blood pressure was defined as mean of the second and third reading of the consecutive blood pressure measurements.

Biochemical evolution

Blood samples were obtained under fasting condition and the following tests were performed using standard laboratory methods. Fasting blood sugar (FBS), Serum cholesterol (T.CHO), Triglycerides (TGL), High density lipoprotein (HDL-C), Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline Phosphatase (ALP) and Serum Albumin. All blood investigations were done on chem. V7 analyzer.

Insulin level was assessed using the method of indirect chemiluminsescence (MEIA). Insulin resistance was calculated according to the HOMA index (Homeostatic Metabolic Assessment).

FBS mmol/dl X insluliniu/L ÷ 22.5[1, 8, 12] or FPI (IU/L) X FBG÷ 405 (FBG in mg/dL)

Statistical analysis

Basal fasting glucose, Insulin, lipid profile and LFT were estimated. Their mean (S.D) were calculated and P- values were done. Pearson's correlation coefficients were calculated to find the type of association between IR and other variables. Finally sensitivity specificity positive predictive value (PPV) and negative predicative value (NPV) were calculated.

RESULTS

Patients attended the outpatient department of Santhiram General Hospital with the visceral obesity and symptoms of liver dysfunction were taken into study (N=150). By applying inclusion criteria we obtained 90 subjects. All these were undergone Ultrasonography. Based on the findings, patients were categorised into two groups, NASH (n=40) and cirrhosis (n=40). Forty age matched healthy subjects (n=40) were taken for control.

Table 1:

Shows the comparisons of metabolic risk factors and Liver function tests in controls and NASH groups. Most of the NASH subjects were fall in between 30 -40 years. Insulin resistance of these subjects was significantly elevated. Liver enzymes were in the Upper reference level. Serum albumin levels were slightly decreased but within the reference level. So this gives an impression that metabolic syndrome is one of the risk factor for NASH.

Table 2:

Shows comparison between metabolic risk factors and Liver function tests of NASH and cirrhotic patients, all the clinical markers of metabolic syndrome were found to be progressively increased from NASH to cirrhosis, except WC which is not considered as significant factor for disease progression. Here albumin levels were below the reference range which shows A/G reversal, indicating liver cell injury. Subjects included in cirrhotic patients were within the age group of 50 to 60 years. There is a significant increase in Dyslipidemia in early stages of liver disease (NASH) followed by a raise of LFT in cirrhosis.

Table 1: Patient characteristics expressed as mean and standard deviation

PARAMETER	*CONTROL (MEAN±SD)*	*NASH(MEAN±SD)*	*P VALUE*
Age	41.96±9.98	41.23±10.43	0.783
WC (cm)	80.83±4.6	127±18.76	<0.001
FBS (mg/dl)	82.2±6.56	127.26±23.02	<0.001
HOMA IR	1.22±0.38	4.67±0.35	<0.001
CHO (mg/dl)	162.7±11.1	216±45.0	<0.001
TGL (mg/dl)	148.1±15.3	257.63	<0.001
HDL (mg/dl)	44.83±3.26	33.13±4.27	<0.001
AST (IU/L)	21.2±3.7	52±8.12	<0.001
ALT (IU/L)	21.3±2.56	50.83±10.19	<0.001
ALP (IU/L)	103.0±13.07	188.66±68.57	<0.001
ALBUMIN (mg/dl)	4.66±0.16	4.07±0.3	<0.001

Table 2: Comparison of biochemical parameters in NASH and Cirrhosis

PARAMETER	*NASH (MEAN±SD)*	*CIRRHOSIS(MEAN±SD)*	*P VALUE*
Age	41.23±10.43	57.66±8.79	<0.001
WC (cm)	127±18.76	136.4±10.57	0.09*
FBS (mg/dl)	127.26±23.02	194.23±58.79	<0.001
HOMA IR	4.67±0.35	9.57±4.41	<0.001
CHO (mg/dl)	216±45.0	314.5±6.72	<0.001
TGL (mg/dl)	257.63	438.1±72.36	<0.001
HDL (mg/dl)	33.13±4.27	28.3±5.2	<0.001
AST (IU/L)	52±8.12	319.13±133	<0.001
ALT(IU/L)	50.83±10.19	325.9±18.91	<0.001
ALP (IU/L)	188.66±68.57	435.03±80	<0.001
ALBUMIN	4.07±0.3	2.56±0.13	<0.001

Not significant

Table 3: Correlation coefficient of parameters with Insulin resistance

Correlation IR vs.		*TGL*	*CHO*	*HDL**	*AST*	*ALT*	*ALP*	*ALBUMIN**
NASH	r-value	0.9342	0.4239	-0.6432	0.4020	0.4027	0.4965	-0.2042
	p-value	0.001	0.015	0.001	0.0267	0.0274	0.005	0.2791
CIRRHOSIS	r-value	0.7659	0.5496	-0.6929	0.6843	0.6895	0.6895	-0.5952
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

negative correlation

Table 4: Evaluation of clinical parameters in NASH

Variables	*Cut off*	*Sn.(%)*	*Sp.(%)*	*LR+*	*LR-*	*PPV(%)*	*NPV(%)*
WC	80	86.67	93.33	13.60	0.14	92.86	87.50
FBS	100	86.67	100	13.33	0.13	100	88.24
HOMA IR	2.5	96.69	96.67	29	0.03	96.69	96.67
CHO	170	100	83.33	6.00	0.00	85.7	100
TGL	150	93.33	86.67	7.0	0.08	87.50	92.86
AST	45	70.00	80.00	3.50	0.38	77.78	72.73
ALT	45	73.33	80.00	3.67	0.33	78.57	75.00
ALP	180	60	80.00	3.00	0.50	75.00	66.61

Sn: sensitivity, sp: specificity, LR: likely hood ratio, PPV: positive predictive value, NPV: negative predictive value.

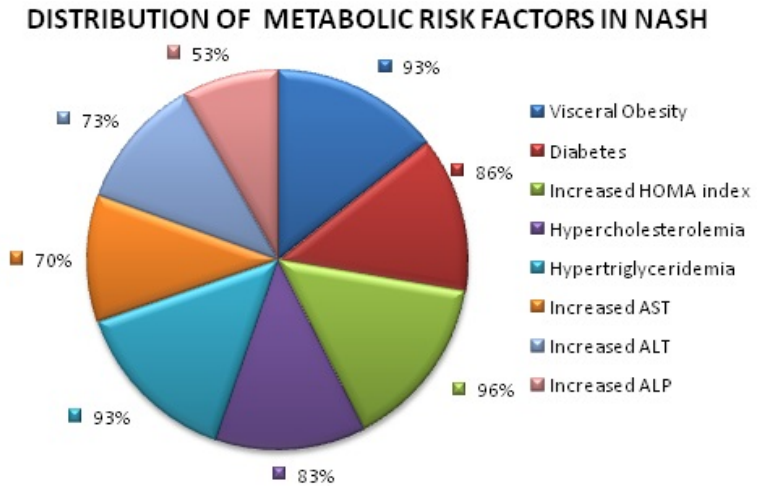

Table 4

Comparatively Dyslipidemia has significant correlation with HOMA IR than LFT in NASH, whereas in cirrhotic patients both Dyslipidemia and LFT were significantly correlated with HOMA IR. A small decrease in triglyceride levels is observed in progression of NASH to cirrhosis.

DISCUSSION:

Non-alcoholic fatty liver disease (NAFLD) is a group of co morbidities ranging from simple liver steatosis to Non-alcoholic steatohepatosis (NASH), which is progressive to cryptogenic cirrhosis [3]. A study conducted at John Hopkins University of Medicine using data from the third National health and Nutrition Examination survey (NHANES III) indicates NAFLD to be cause of up to 84% of all liver abnormalities [4].

NASH was thought to be a benign disease but unfortunately this perception has persisted despite accumulating evidence that NASH is now the most common form of chronic liver disease. The best estimates of this can be studied by inverted NAFLD pyramid, about 20 % adults have excess fat in the liver shown here as an inverted pyramid. Of these people, 10% to 15 % have NASH and 20% of those with NASH are at risk for developing cirrhosis, up to 30 %to 40%. Of those with NASH cirrhosis will die from end stage liver disease. This is associated with vascular disease, complicated diabetes, massive obesity and these patients are ineligible for transplantation[5]

Increased visceral obesity develops resistance to insulin, causes deregulation of adipose derived fatty acid flux, leads to excessive accumulation of lipid in hepatocytes called Fatty Liver - a hall mark in NAFLD [6]**.** Insulin resistance has associated with a chronic subclinical inflammation and characterized by increased C-reactive protein [7], interleukin (IL)-6, IL-8, monocyte chemotactic protein (MCP)-1, and tumour necrosis factor (TNF-α) have also been found to be increased with elevated insulin resistance [8-10,] each directly impair insulin sensitivity by interfering with insulin stimulated glucose uptake in peripheral tissues causes elevation of Fasting blood glucose levels[11-13].

Visceral obesity is one of the most important risk factors for NASH [14]; waist circumference remains the simplest and most widely used parameter. In our study all most all patients (86%) had increased waist circumference with a mean value of (127.83±18.76). We evaluated the predictive role of waist circumference for NASH diagnosis (92.86%). Singh etal identified WC as an independent predictor for the degree of liver necroninflamation [15]. Park et al used the clinical BMI value to differentiate the simple steatosis from NASH, establishing a threshold value for BMI as indicator for NASH [16].

Insulin resistance is recognised as a major determinant of steatogenisis and possibly of liver disease progression. In our study NASH group presented with higher FBS and Insulin Resistance (IR) and Dyslipidemia with Liver Function tests in their upper normal limits with a decreased HDL levels (table 1). HOMA IR was correlated with all the clinical markers of metabolic syndrome and was found to have a positive significant correlation in NASH (table 3). This is in accordance with Carmen Fierbinteanu-Braticevici etal study on Predictive Factors for NASH in NAFLD patients[17]. A study done by Leevy in NAFLD Patients reported that NAFLD was related to obesity, Dyslipidemia, diabetes mellitus[18]**.**

The predictive factors for progression of NASH were studied by correlating HOMA IR with metabolic risk factors in cirrhotic patients (table 2). Our data found that IR is greatly elevated correspondingly with an elevation of lipid profile and liver function tests. We found A/G reversal in cirrhotic patients, which is a strong predictor for fibrosis. This proves the statement that insulin resistance (IR) as a major determinant of steatogenisis and possibly of liver disease progression [19].

NASH is not associated with any characteristic symptoms and it is a reversible disease. Diagnosis of NASH in NAFLD requires a liver biopsy and ultra- sonography imaging techniques. Laboratory testing can provide some clues to the presence of NAFLD. Serum aminotransferases are most commonly used test to screen unsuspected liver diseases such as NAFLD. Unfortunately these lack specificity and sensitively for NASH. At this time WC and clinical markers of insulin resistance were found to be elevated in NASH[20, 21]. In our study we found positive predictive value (100%) for FBS, followed by IR (96.69%), WC (92.86%), and TGL (87.50%) in decreasing order. Our data also shows highest specificity for FBS (100%), followed HOMA IR (96.67%), WC (93.33%) and TGL (86.67%) (Table: 4) in accordance with the study done by Carmen Fierbinteanu-Braticevici etal.

CONCLUSION

The central role of insulin resistance in pathogenesis of NASH suggests that interventions directed at improving insulin sensitivity might be beneficial in stopping disease progression or reversing established disease. A simple non invasive predictive model that incorporates both clinical and biochemical parameters of metabolic syndrome can identify patients at risk with NASH at an early stage and the condition can be reversed by taking proper therapeutic measures, avoiding routine liver biopsy.

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