Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 4161 - 4168

Received 15 December 2020; Accepted 05 January 2021.

"Interleukin-six" As Predictor of "Non Alcoholic Fatty Liver Disease" among "Insulin Resistance Syndrome" : A study protocol

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Type of Article- Study Protocol
Conflict of Interest: **None**
Funding: Nil .

Abstract:Background: "Non Alcoholic Fatty Liver Disease" (NAFLD) Patients may progresses to cirrhosis of livercirrhosis and decompensated liver cirrhosis if the risk factors of like "Insulin resistance syndrome", obesity, diabetes are not controlled. Interleukin six is one of the predictors of NAFLD progression.

AIM: To find out the role of "Interleukin-6" as predictor of "Non-alcoholic Fatty Liver Disease" among "Insulin Resistance Syndrome" and to correlate the level of IL-6 with Anthropometric profile, lipid profile Fasting Blood glucose, NAFLD score, and ultrasound of liver.

Methodology: The study will be conducted in medicine department of AVBH ,Sawangi (Meghe) which is affiliated to DattaMeghe institute of Medical sciences, Wardha. It will be aObservational, Case Control study. Patients height, weight, blood pressure, neck circumference, waist and hip circumference, biochemical investigations like fasting plasma glucose, Fasting Lipid Profile,LiverFunction Tests,PlateletCounts and Interleukin six will be considered to ascertain the patient of "InsulinResistanceSyndrome", and estimate the NAFLD

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score using above parameters and correlate it with USG of liver findings and predict the correlation between scoring, IL 6 and radiological findings .

Expected Results and Discussion: Among patients with non-alcoholic steato-hepatitis (NASH), IL-6 level will be higher relative to simple steatosis, assisting the existence of hepatic IL-6 expression in human NASH.We may find out positive association between the expression of IL-6 and NAFLD score and ultrasound findings. Author may find association of IL-6 levels with systemic "Insulin Resistance Syndrome".
In animal models of NAFLD, the hepatic expression of "Interleukin-6" (IL-6) is increased, whereas in mice, selective sustained overexpression of IL-6 results in systemic resistance to insulin.As per above animal study,we hypothesis that il-6 may be used as a predictor of progression of NAFLD..
Keywords: "Non Alcoholic Fatty Liver Disease" (NAFLD) , "Interleukin-6" and "Insulin Resistance Syndrome".

INTRODUCTION:

"Non-alcoholic fatty liver disease" (NAFLD) is leading cause of chronic liver disease in India. Nowadays, liver biopsies in India are the only benchmark for NAFLD identification**(1)**. The liver is the main organ in fat metabolism and also forms the homeostasis of lipid metabolism. The liver synthesize (lipogenesis) or reserves fatty acids as per the needs of the body.This process of lipid homeostasiss is controlled by hormones such as insulin and glucagon are involved. An abnormal accumulation of lipids contributes to fatty liver disease when these controls are disrupted**(2)**. This mechanism is generally referred to as steatosis. Consumption of alcohol is one of the main source of fat homeostasis; disturbance in the liver. When unhealthy lipid deposition in the liver occurs due to other causes which are not in relation with the alcohol consumption; it is known as "Non-alcoholic fatty liver disease"**(2)**. Other causes of "Non-AlcoholicFattyLiverDisease" are i) Nutritional: starvation, obesity, bariatric surgery, parenteral nutrition, celiac disease ii) Metabolic: dyslipidemia, insulin resistance, fatty liver of pregnancy iii)Drugs: tamoxifen, amiodarone, valproic acid, zidovudine, glucocorticoids. iv) Others: inflammatory bowel disease, toxic mushroom consumption .Non-alcoholic fatty liver disease (NAFLD) has been the leading cause of chronic liver disease worldwide, along with the increasing prevalence of obesity, diabetes mellitus and metabolic syndrome in the general population **(3-6).**

PATHOGENESIS OF NAFLD:

1.Obesity in the liver contributes to a rise in free fatty acids
2. "Non Alcoholic Fatty Liver Disease"
3. Non Alcoholic Steatohepatitis

There is a complex relationship between environmental factors which is involved in NAFLD pathogenesis such as diet, obesity, microbiota modifications and predisposing genetic factors, resulting in impaired lipid homeostasis and unrestricted accumulation of triglycerides and other lipid species by hepatocytes.Insulin resistance is a one of the key mechanism that involves in lipid toxicity, endoplasmic reticulum stress, impaired autophagy, and ultimately liver cells damage and death, leading to hepatic inflammation, activation of hepatic stellate cells and progressive fibrogenesis, leading to disease progression.In this analysis, we sum up the data available on NAFLD pathogenesis, highlighting the most current findings. For the development of new and successful therapeutic methods, increased awareness of NAFLD/NASH pathogenesis is important.

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Newer Biomarker Research in NAFLD and NASH: various type of biomarkers and hormones, pro-inflammatory cytokines and proteins, adipokines .They are main targeted subject for researchnowadays, for the identification, classification, and monitoring of NAFLD and NASH**(7).**

Current Biomarkers of Interest are For NAFLD: ADP,Leptin,CRP,TNF-alpha,Interleukin-6

Current Biomarkers of Interest areFor Non Alcoholic Steatohepatitis : ADP, TNF- alpha, Leptin, "Interleukin-6"

Current Biomarkers of Interest areFor Fibrosis: ADP, Leptin

Interleukin-6 (IL-6)

'Interleukin 6'(IL-6) is a single form of pro-inflammatory cytokine that is released in a state of insulin resistance. Previous research indicates that, relative to controls, extreme high serum IL-6 levels are observed in NAFLD patients**(9)(10)**. Among NASH patients, IL-6 levels are positively correlated with hepatocyte inflammatory response, progression to fibrosis, and associated Insulin Resistance Syndrome**(11)**.

In the advancement of NAFLD to NASH, there is a pro-inflammatory cytokines "interleukin-6"(IL-6) according to data from mouse models. A high fat diet in mice results in increase of IL-17 and TNF-alpha by liver macrophages.

NAFLD Fibrosis Score:

The NAFLD Fibrosis is result method of non-invasive outcome depend on many clinical tests that help to measure the quantity of liver scarring. NAFLD in liver disease has studied only by using this score. Components of NAFLD Score: (1) AST 2) ALT (3) Age (4) BMI (5) Albumin (6) Platelet Count (7) Impaired Fasting Glucose/Diabetes**.(8)**

There are very few studies done regarding pro-inflammatory biomarkers of NAFLD and NASH in India. Hence we proposed the study to evaluate role of interleukin-6 as prognostic marker of non-alcoholic fatty liver disease.

AIM: To determine the role of "Interleukin-6" as predictor of "Non-alcoholic Fatty Liver Disease" among "Insulin Resistance Syndrome".

Objectives:

(1) To determine the level of interleukin-6 level among "Non Alcoholic Liver Disease".

(2) To determine the NAFLD Score among study population.

(3) To correlate the level of IL-6 with Anthropometric profile, lipid profile AND Fasting Blood Glucose.

Material and Method

Setting:

The study will be conducted at the AcharyaVinobaBhave Rural Hospital (AVBRH), a teaching tertiary care hospital located in the Sawangi (Meghe) Wardha rural area of Central India.

Duration of Study:

The duration of study will be from October 2020 to November 2022

Study Design:

Observational case control study.

Participants:

Study participants will be the patient attending medicine OPD or admitted to medicine Ward.Ultrasound of the abdomen to find out fatty liver disease in patients at high risk of insulin resistance syndrome 'insulin resistance syndrome' IRS (Obese, Diabetes and Metabolic Syndrome) insulin resistance syndrome.Insulin resistance syndrome (IRS) will be described as per guidelines of who.

Inclusion criteria of cases:

Received 15 December 2020; Accepted 05 January 2021.

1. All Patients above 18 years
2. According to WHO consultation (1999) "Insulin resistance syndrome" is described as person having Diabetes Mellitus or impaired glucose tolerance, together with 2 or more of the following:
"(1)Body Mass Index (kg/m2) ≥30
(2)Waist to hip ratio : >0.9 (M), >0.85 (F)
(3)Arterial pressure(mm Hg) : ≥140/90
(4)Triglyceride (mg%): ≥150
(5)'DL-cholesterol (mg%): <35 (M), < 39 (F)
(6)"Microalbuminuria" ≥20μg/min"

Control: age(+/-) and gender matched control selected

Exclusion criteria:
1) Pregnant women
2) Patient not giving consent
3) Patient taking drugs like steroids,tamoxifen,valproate,zidovudin
4) Patient having chronic diarrhoea

Sample size:
200
n=Zα/2
2 * P * (1-P) / d2
Zα/2 is the level of significance at 5% i.e. 95% confidence interval = 1.96
P = prevalence of Refractory heart failure 1.2% = 0.012
d = Desired error of margin = 3% = 0.03
n = 1.962 X 0.012 X (1- 0.012)/0.032
n = 50.60 = 55 patients needed in the study

Methods:
Patient will be recruited from the medicine ward after seeking Written informed consent. A full clinical history, socio-demographic data will be collected. Anthropometric measurements of the participants like Body Mass Index (BMI), Waist Circumference (WC), Head Circumference(HC) , Neck Circumference(NC), Waist-to-Hip Ratio (WHR) measurement will be done as per WHO criteria**(12)**.Estimation of Body Mass Index is done with the division of weight (kg) by the square of height(m), and Waist-to-Hip Ratio is defined as the division of perimeter of the waist (cm) by the perimeter of the hip (cm). The fasting blood glucose will be measured after 8 hours ofovernight fasting. Other biochemical profile such as FLP(fasting lipid profile), LFT(Liver Function Test), KFT(Kidney Function Test) platelet count and Il-6 level estimation. Evaluation of Interleukin-6 in serum will done by using ELISA with the directive of company, ultrasound of the abdomen will be done for the diagnosis of fatty liver disease .NAFLD fibrosis score will be calculated for both groups(Clementia Biotech). All the above parameters will be done by NABH Approved laboratory.

Some of the measuring tools for assessment of obesity
Body Mass Index (BMI): The BMI is the person's weight (kilogram) divided by square height (meter). The high BMI score shows high body fat, and the low BMI score shows low body fat.

- **BMI value < 18.5 =** underweight
- **BMI value 18.5 - 24.9 =** normal or Healthy Weight range

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- **BMI value 25.0 - 29.9 =** comes under overweight
- **BMI more than 30.0 =** within obese range.

For a given height, overweight or obesity is described as a weight that is greater than what is considered as a healthy weight. Underweight for a given height is defined as a weight that is smaller than what is considered acceptable.

BMI may be used at an individual level as a screening instrument, but is not diagnostic of the body fat or health of an individual. A trained healthcare professional should perform comprehensive health assessments in order to determine a person's health status and risks.

Neck Circumference:

Neck circumference is a basic screening test for the detection of overweight and obese patients. Neck circumference (NC) has been suggested in numerous studies in the past as a useful predictor of upper body obesity**.(13,14,15,16)**These studies found that males with NC < 37 cm and females with NC < 34 cm had a low body mass index.(**13)**

In both sexes, NC was measured just below the laryngeal prominence (Adam's apple) to the nearest 0.1 centimeter. With the subjects standing erect, with shoulders relaxed using flexible measuring tapes, all circumferences were taken.

Study findings on neck circumference as a predictor of obesity and overweight in rural central region showed that the correlation coefficients of Pearson showed a strong association between NC changes and body mass index changes. BMI is positively correlated with NC (Corr.coeff = 0.59, $p < 0.01$) and weight (Corr.coeff = 0.60, $p < 0.01$) in males. BMI was also positively associated with NC (Corr.coeff = 0.74, $p < 0.01$) and weight (Corr.coeff = 0.82, $p < 0.01$) in women. The ROC analysis showed that for NC and BMI >25 kg/m2, the area under the curve (AUC) was 0.89 for men and 0.91 for women, respectively. The best cut-off points for assessing subjects with overweight is NC ≥38 cm for men and ≥ 34.7 cm for women. Men with NC < 36.6 cm and women with NC < 32.1 cm should therefore not be considered overweight. Additional measurement of overweight or obese status is needed for patients with NC > 36.6 cm for men and > 32.1 cm for women.

Classification of NAFLD patients will be done into two subgroup according to intensity level of fibrosis which is based on 6 variables as described.

Fibrosis score between -1.455 and 0.676 = classified as mild fibrosis

Fibrosis more than 0.676 = classified as moderate or significant fibrosis.

The comparison of results will be done, among study participants.

Definitions:

1. Fatty liver definitions: Fatty liver is diagnosed based on following USG parameters

a) Brightness of parenchyma

b) Contrast from Liver to kidney

c) Deep beam attenuation

d) Bright vessel wall

e) Gall bladder wall definition

2. Normal IL-6 level: 0-16.4 pg/ml Increased level >16.4 pg/ml

3. NAFLD Score Formula:-"1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×109/l) – 0.66 × albumin (g/dl)"

Explanation of Result:

"NAFLD Score less than -1.455 = F0-F2"

"NAFLD Score between -1.455 and 0.675 = indeterminate score"

"NAFLD Score more than 0.675 = F3-F4"

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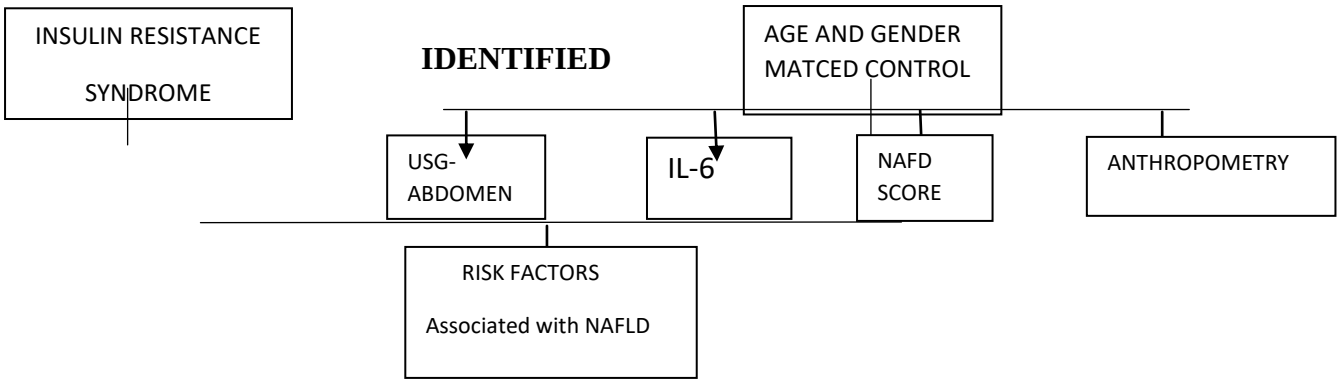
Expected Results



(1) We will be able to find out the prevalenceof "Non Alcoholic Fatty Liver Disease" among "Insulin Resistance Syndrome" group and among control group by performing USG of Abdomen and Pelvic.
(2) We will be able to find out the prevalenceof "Non Alcoholic Fatty Liver Disease" among "Insulin Resistance Syndrome" group and among control group by performing serum "Interleukin-Six" level.
(3) We will be able to find out the Risk Factor associated with "Non Alcoholic Fatty Liver Disease"

DISCUSSION:

The relationship between the metabolism of liver fat and pro-inflammatory circulating levels is a complex patho-physiological mechanism that is not well understood. This inevitable phase could be curtailed at the early stage if NAFLD progression to NASH and Liver cirrhosis were detected early. A number of related studies were reviewed(17-19). Dangoreet. al. assessed the risk of liver fibrosis in areca nut habitual by ultrasound elastography (20). Aryaet. al. reviewed association between cardiac dysfunction, arrhythmias and chronic liver diseases (21). Baggaet. al. explored the spectrum of non -alcoholic fatty liver disease (NAFLD) in nursing staff (22). Related studies were also reported by Hussain et. al.(23) and Sawarkar et. al. (24).

The proposed study will identify the role of "Interleukin-6" in identifying the progression of "Non Alcoholic Fatty Liver Disease" to "Non Alcoholic Stetohepatitis" early on.There are some disadvantages to this analysis that need to be taken into account. Second, it will be conducted in a small group of patients; this is primarily due to the high cost of the assays used in this study. In addition, there was no stable individual control group and all the people studied displayed a degree of non-alcoholic fatty liver disease," which limits the complete analysis of the findings and further extrapolation. The main caveat in this approach is that as they overlap in the analysis, it is not possible to fully establish which characteristics may certainly be correlated with which of the three modes of liver disease are considered. Finally, a variety of mediators that have not been analyzed in this research are involved in the complete inflammatory panel; as there is a complex relationship between these substances, the final conclusions can also be avoided. Despite these caveats, the results of the current study provide useful insights into the development of liver disease in people with obesity and increase the likelihood of using any of these molecules as diagnostic and follow-up markers or as future therapy targets.

CONCLUSION:

In this study we are expecting increased serum IL-6 production may find main key factor in NASH development and progression to cirrhosis, as well as in systemic insulin resistance. It

is clear that in order to provide extra resources to investigate and monitor the development of NAFLD, alternative screening approaches need to be researched and accepted. Emerging NAFLD and NASH biomarker studies will open the way for new procedure and future treatments that are not invasive.

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