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RESEARCH ARTICLE

Metabolic Syndrome (MetS) and Nonalcoholic Steatohepatitis (NASH)

Study of biochemical markers Free Fatty Acid (FFA), Total Antioxidant Status (TAOS), Adiponectin, Transforming Growth Factor (TGF-ß1), in occurence of NASH

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BACKGROUND: The prevalence of metabolic syndrome (MetS) in USA and Makassar are 22% and 23.7%. The prevalence of Non Alcoholic Steatosis Hepatosis (NASH) in MetS has not been reported. Study in Non-alcoholic Fatty Liver Disease (NAFLD) is 25–90 % in obesity patients. In NASH, there is accumulation of lipid in hepatocyte (raised free fatty acid level), raised stress oxidative (decreased total antioxidant status), raised of inflammation process (decreased adiponectin) and hepatic fibrotic process (raised TGF β1). The aim of this study is to investigate the correlation of free fatty acid, total antioxidant status, adiponectin and TGF-β1 with the occurrence of NASH.

METHODS: This was a case control study in man aged ≥ 30 years old. Metabolic syndrome (MetS) was defined by IDF categories. NASH was defined as fatty liver plus raised type IV collagen level ≥ 140 ng/ml and Alanine Transferase (ALT) level 1.5 X upper normal limit.

RESULT: The samples consisted of 8 MetS subjects, 11 MetS subjects with fatty liver and 2 MetS subjects with suspect NASH. Low level of adiponectin and high level free fatty acid led to progression from Fatty Liver (FL) to NASH. Level of total antioxidant and Level of TGF-β1 were relatively steady in NASH.

CONCLUSION: The level of Free Fatty acid in subjects with MetS-FL was higher than in subjects with MetS, but was lower than in subjects with MetS-NASH. No difference in total antioxidants status level was observed among all groups. Level of adiponectin decreased in subjects with MetS-FL and MetS-NASH compared with subjects with MetS only. The level of TGF-β1 increased in subjects with MetS-FL more than in subjects with MetS only, and was steady low in subjects with MetS-NASH.

KEYWORDS: Metabolic Syndrome, NASH, Free Fatty Acid, Total Antioxidant Status, Adiponectin , Transforming Growth Factor β1.

The prevalence of metabolic syndrome in USA and in Makassar are 22% and 23,7% respectively. Individuals with metabolic syndrome seemingly are susceptible to other conditions such as Polycystic Ovary Syndrome (PCOS), fatty liver, asthma, sleep disorders and some form of cancers (1).

Nonalcoholic steatosis hepatitis (NASH) is defined by the presence of hepatomegaly, increase of aminotransferase serum and lacked history of significant alcohol intake but in whom liver histology resembled that of alcoholic liver disease (2). NASH—the most severe form of non-alcoholic fatty liver disease (NAFLD)—is emerging as a common clinically important type of chronic liver disease industrialized countries. Most cases of NASH occur in patient with obesity (25–95%), type 2 diabetes mellitus (21–55%) and hyperlipidemia (23–92%). There was no data of occurrence of NASH in metabolic syndrome (3).

Insulin resistance and increased free fatty acid level were major features in pathomechanism of NASH. Insulin resistance and hyperlipidemia caused lipid accumulation such as triglyceride, hepatocytes that were more supceptible to oxidative sress, cytokines expression, inflammation, and decrease in ATP triggering hepatocyte injury (4).

Inflammation, a combined process of stress oxidative effect, lipid peroxidation and abnormally cytokine expression especially TNF-α, mediated the occurrence of

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intrahepatic inflammation, apoptosis, necrosis and fibrosis of hepatocytes. It also increased the production of TGF-β1, which could activate the steallate cells and caused cell death (4). The liver normally responds to the chronic presence of oxidant pathway, such as those based on reduced glutathione (GSH) and total antioxidant (5)

This study investigated the biochemical markers of Free Fatty Acid (FFA), Total Antioxidant Status (TAOS), Adiponectin, Transforming Growth Factor β1 (TGF-β1), in the occurrence of NASH.

Patients and Methods

This study was an observational case control study. Metabolic syndrome (MetS) was defined by IDF categories as waist circumference plus any two of the following factors: raised triglyceride level ≥ 140 mg/dl, reduced HDL cholesterol < 40 mg/dl (in man), raised blood pressure (systolic ≥ 130 or diastolic ≥ 85 mmHg), raised fasting plasma glucose ≥ 100 mg/dl. NASH was defined as fatty liver plus raised type IV collagen level ≥ 140 mg/dl and ALT level 1.5x upper normal limit.

Subjects consisted of 8 Mets subjects, 11 MetS subjects with fatty liver and 2 MetS subjects with suspect NASH. Informed concent from all subjects were obtained before subjects were enrolled in this study.

Assay of Biochemical Markers

Serum were separated from fasting whole blood after centrifugation, and immediately kept at -20ºC until measurement. Biochemical markers measured were free fatty acid (Roche,cat.No. 11383175001), TGF-β1 (R&D, Cat.No. DB100), Total antioxidants status (Cat. No. NX2332), Adiponectin (Daichii Cat.No. 376405 lot 006RED), and collagen type IV (Daiichi, Cat No. 348259 Lot No.CH01401).

Statistical Analysis

SPSS for windows ver 11.5 was used for all statistical and graphical analysis. General description of data were analyzed with univariate analysis to mention minimum and maximum value, average, and standard deviation. T-test was used to compare all factors between subjects with MetS-NASH and subjects without MetS-NASH. Bivariat and partial correlation analysis was used to analyze correlation of all factors. Results were considered significant if $P \leq 0,05$.

Results

1. FREE FATTY ACID

In subjects with MetS progressing into Fatty liver, there was a significant increase in FFA level, while in the subjects with MetS progression into NASH, the level of FFA tended to decrease, as shown in Figure 1.

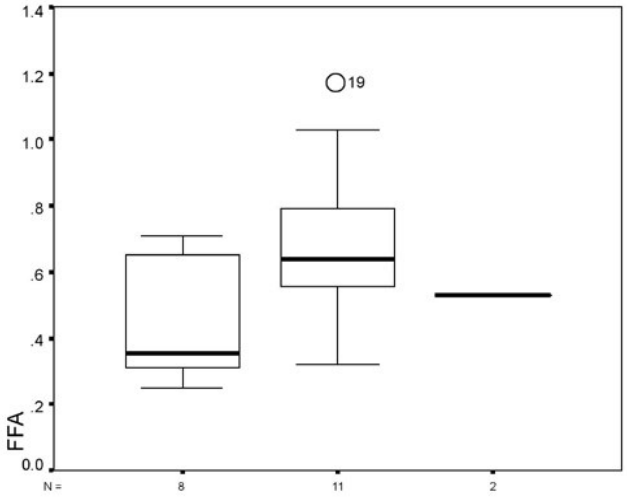

Figure1. Means of free fatty acid level in Mets, Mets With Fatty Liver and Mets with NASH

2. TOTAL ANTIOXIDANT STATUS

In this study, we found no significant difference in the level of total antioxidant status in subjects with MetS and in subjects with MetS-Fatty liver, as can be seen in Figure 2. This might be due to the measured antioxidant was not an active antioxidant that played role in the pathobiology of NASH or either because the antioxidant involved in NASH, worked simultaneously and thus did not show any significant level difference.

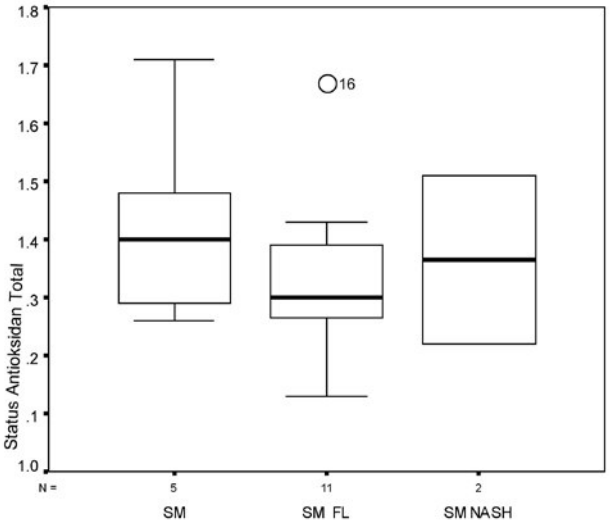

Figure . Means of free total antio idant status level in Mets, Mets With Fatty Liver and Mets with NASH

3. ADIPONECTIN

Figure 3 showed that the subjects with MetS-FL or MetS-NASH had lower level of Adiponectin compared to subjects with MetS only.

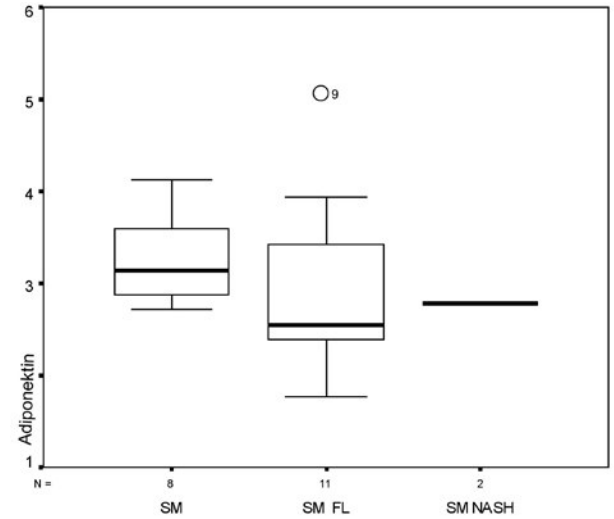

Figure 3. Means of free adiponectin in Mets, Mets With Fatty Liver and Mets with NASH

4. TRANSFORMING GROWTH FACTOR β-1 (TGF-β1)

In subjects with MetS progressing into fatty liver MetS, there was a significant increased level of TGF-beta1, however in subjects with MetS-NASH it tended to decrease, as seen in figure 4. This might be due to the role of TGF-beta1 that only took place in the early stage of MetS to Fatty liver MetS. In the stage of MetS-NASH, this role might be displaced by other markers or collagen tissue might have already formed and thus lesser TGF-beta1 could be detected.

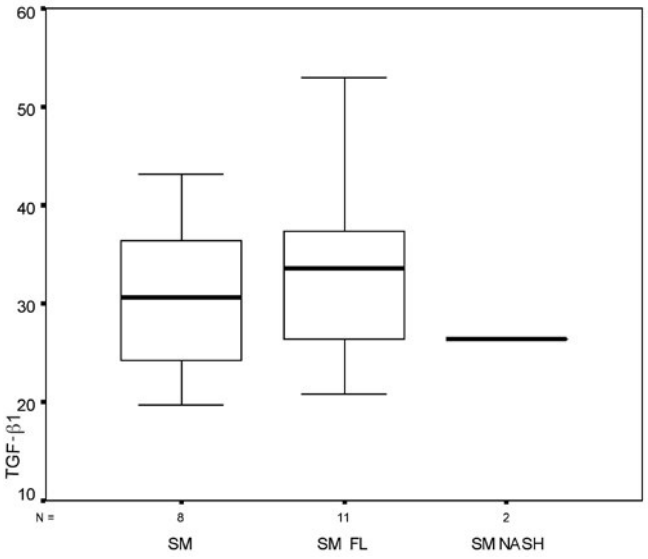

Figure 4. Means of TGF-ß1 level in Mets, Mets With Fatty Liver and Mets with NASH

Discussions

Histopathologically, NASH is a part of a wide spectrum of Nonalcoholic fatty liver disease which found as a simple steatosis up to fibrous steatosis (Ludwig, 2000) We defined NASH with combined measurement of USG, SGOT, and Collagen type IV. Resulted conditions (Mets, Fatty liver MetS, MetS suspect NASH) were probably due to a process that started from NAFLD up to NASH, and these were proved when we diagnosed the type of NASH by using liver biopsy with steatosis spectrum, steatohepatitis, and NASH.

There are several parameters that play roles in NASH pathomechanism, such as FFA, total antioxidant status, adiponectin and TGF-beta1.

The level of FFA decreased in MetS-NASH and this might be due to cellular progressivity that happened since fatty liver MetS until NASH MetS and therefore FFA was

oxidized (peroximal beta oxidation or m icrosomal W oxidation or it was esterified into triglyceride, see figure 5). The changes in FFA concentration in these three conditions were further observed with a larger number of samples.

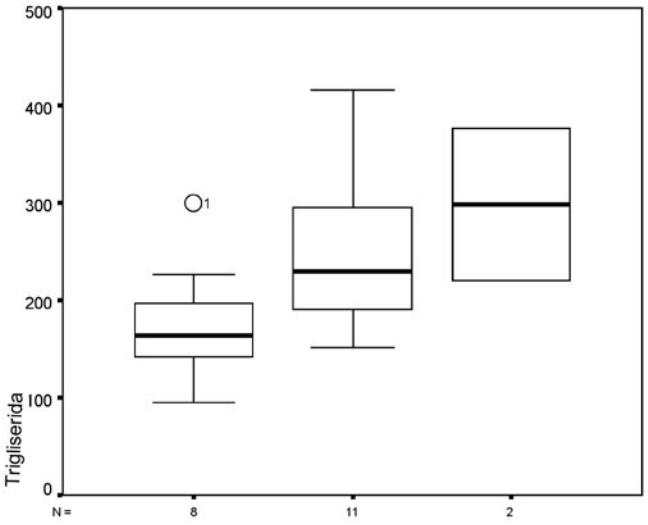

Figure 5. Means of triglyceride level in Mets, Mets With Fatty Liver and Mets with NASH

Reactive oxygen s pecies causes a decrease in antioxidant enzymes, the measurement of antioxidant status represents the oxidative status in subjects with MetS along with Fatty Liver.

However, to prove this hypothesis, further research using larger s ample number and a lso a c omparison using other antioxidants such as superoxide dismutase (SOD) is still needed. The evaluation of stress oxidative condition can also be done using thioredoxin.

Obesity and M etS will r esult in a n increased o f reactive oxygen species and altered lipid peroxidation and therefore result in inflammation that triggers the release of cytokines such as TNF-alfa and TGF-beta1, and a decreased in adiponectin level.

From the study of Yoon *et al*, hypoadiponectinemy occurred i n NAFLD s ubjects. I n animal study, adiponectin w as p rotective towards the alcoholic effect a s well a s nonalcoholic f atty liver d isease. The recent study, has r eported that hypoadiponectinemy was a picture of NASH that was non insulin resistance dependent and t hat t he decreased adiponectin l evel correlated t o the number o f necroinflammation and c ontributed t o the progression of t he s hape o f necroinflammation in NAFLD (Yoon, 2005).

Obesity also causes an increase of reactive oxygen species and a ltered lipid p eroxidation and t hus triggering inflammation which is marked by the release of c ytokines s uch as T NF-alfa and T GF-beta1 and a decrease of adiponectin. The continuous induction of TGF-beta1 will result in the formation of fibrous and it is marked by the increased level of collagen type IV.

The f ull pathomechanism o f interaction of F FA, adiponectin, total antioxidant status, TGF-beta1 and collagen type IV can be seen in figure 6.

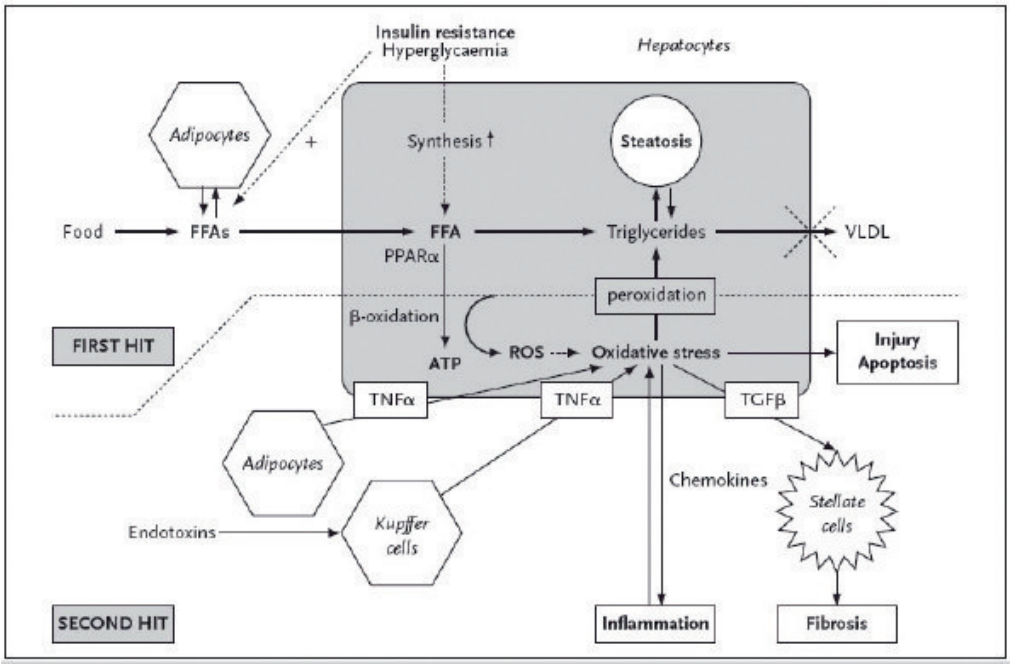

Figure 6. From Fat to liver injury (Adapted from Jansen, 2004).

Free fatty acid are taken up in the hepatocytes where they are either metabolized via peroxisomal and mithochondrial β-oksidation or stored as triglycerides. VLDL is the rate –determining step in triglyceride export from the liver. In NASH its synthesis is decreased. The insulin-resistant state favours lipolysis in the adipose tissues, FFA synthesis and lipogenesis in the liver. The net result is hepatic fat inflammation. Continuous and superfluous FFA oxidation causes the generation of ROS in excess with oxidant stress as a result. Cytokines are produced in hepatocytes as well as in Kupffer cells. TGF-β stimulates hepatic stellate cells to produce collagen and cause liver fibrosis, in addition these cells transform into myofibroblasts with more collagen formation and an increase of intrahepatic vascular resistance and portal hypertension. Chemokines attract monocytes and neutrophils with inflammation, more oxidant stress, hepatocellular apoptosis and injury as a result

Conclusions

The level of Free Fatty Acid in subjects wit MetS-FL was higher than in subjects with MetS, but was lower than in subjects with MetS-NASH. No difference in total antioxidant status level was observed among all groups. Level of adiponectin decreased in subjects with MetS-FL and MetS-NASH compared to subjects with Mets only. Level of TGF-β1 increased in subjects with MetS-FL more than in subjects with MetS only, and was steady to low in subjects with MetS-NASH.

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