Anthropometric and Biochemical Characteristics of Patients with Nonalcoholic Fatty Liver Diagnosed by Non-Invasive Diagnostic Methods

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ABSTRACT

Introduction: Non-alcoholic (NAFLD) encompasses a spectrum of disease states, from steatosis (fatty liver) to non-alcoholic steatohepatitis (also called NASH steatosis with inflammatory changes) followed by progression to fibrosis and cirrhosis and hepatocellular carcinoma. Excess liver fat is believed to be a manifestation of the metabolic syndrome and not surprisingly NASH is associated with obesity, insulin resistance, dyslipidemia and type 2 diabetes in humans. Aim of the study: is to establish anthropometric and biochemical specificities in patients with non-alcoholic steatohepatitis diagnosed with non-invasive diagnostic methods.

Material and methods: Study enrolled 170 participants, 130 with NASH steatosis. The non-alcoholic group (control), consisted of 40 normal weight patients without metabolic syndrome. Alcohol intake was estimated with established protocol. Routine biochemistry analysis were performed by standard laboratory procedures; serum levels of fasting cholesterol and triglycerides, fasting glucose and insulin, insulin resistance estimated by HOMA index (Homeostasis model assessment), biochemistry tests and a liver ultrasound examination.

Results: In study participants group, patients were more obese comparing with controls p < 0,01, waist line extent also was of greater statistical significance in the non-alcoholic group fatty liver (p < 0,01). Comparing biochemical parameter values, significant statistical deference has been noted in glaucosis and insulin levels, total cholesterol and gama-glutamil transferase levels, between groups (p<0,01). Fasting glucose and insulin levels, HOMA-IR were significantly greater in study cohort group patients, as was significantly positive correlation between BMI and waist line extent. Conclusion: Patients with non-alcoholic fatty liver are excessively obese, have greater waist line extent, consequently insulin resistance and impaired glucose metabolism, insulin resistance, dyslipidemia, risk factors known to be associated with the development of cardiovascular disease.

Key words: non-alcoholic fatty liver, obesity, body mass index, waist line extent, insulin resistance.

1. INTRODUCTION

The definition of nonalcoholic fatty liver disease (NAFLD) requires that (a) there is evidence of hepatic steatosis, either by imaging or by histology and (b) there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders (1). High serum triglyceride levels, low serum HDL levels and consequent presence of hepatic steatosis, are very common in patients with NAFLD, (histology verification >5% hepatocytes) (2). In summary, estimates of the worldwide prevalence of NAFLD in individuals with dyslipidemia attending lipid clinics was estimated to 6, 3% to 33%, with a median of 20% in general population, based on a variety of assessment method (2). In patients with severe obesity undergoing bariatric surgery, the prevalence of NAFLD can exceed 90% and up to 5% of patients may have unsuspected cirrhosis (3). Obesity is very common and well documented factor of risk for NAFLD. Parameters for the degree and obesity type estimation, excessive BMI Body mass index) and visceral obesity, defined as waist line, are recognized as factor of risk for NAFLD (4).

Insulin resistance (IR), common factor that links obesity, diabetes, hypertension and dyslipidemia with fatty liver leading into progressive form of liver disease, steatohepatitis, fibrosis cirrhosis and hepatocellular carcinoma.

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and mortality. So, liver imaging is the common test used in liver steatosis screening (2). Imaging reveals lever of the homogenic structure, light with increased echogenicity (glittering liver) (15).

Aim of this study was to establish anthropometric and biochemical specificities for patients with non alcohol fatty liver, diagnosed by noninvasive diagnostic procedures.

2. MATERIAL AND METHODS

Prospective study conducted during 24 months (2009-2011) covered and analyzed 170 participants through clinical monitoring. Diagnosis of “non alcoholic fatty liver” confirmed by noninvasive imagining liver ultrasound procedure and by the AST/ALT ratio (1, 2).

Respondents were distributed in two groups:

- Study group (consisted of responders with imagining confirmed fatty liver and AST/ALT pathological values. Group numbered 130 participants.
- Controls (consisted from responders of the same age, slim with imagining that match normal liver findings, 40 of them.

Patients have been monitored in internists ambulances of the Clinical-Hospital Center of Prishtina, and through Health Centre of Gracanica, with already estimated protocols that included patient’s name, generalities and valid anamnestic data. By those protocols anthropometric measurements have been carried out in order to estimate the degree of the nutritional status, mass, and the fatty tissue distribution.

Building up databases and processing was done in SPSS program version 10.0. Also standard descriptive methods were performed, as were analytical methods, t-test for related samples, t-test for independent samples and χ² test. Correlation between biochemical and anthropometric characteristics tested with Pearson's correlation differences of p<0.05 taken as statistically significant.

3. RESULTS

Basic data on the respondents are listed on table 1. There were no big difference among respondents considering age, the average age of the study group was 56, 26 ± 4, 2 years and 56, 40 ± 4, 22 years in control.

In group with nonalcoholic fatty liver there were 78% of man or 60%, and 52 women or 44%, while controls consisted of 24 men or 60% and 16 women or 40%. Between study group and control group no statistical important difference between gender representation could be noted.

Table 1 shows the anthropometric characteristics of the subjects with NAFLD and control group also clinical and biochemical characteristics of the tested groups. Respondents of the study group were more obese at the level of significance compared to the controls, p < 0.01 (BMI (kg/m²)) (32.22±5.50 vs 24.67±3.83), waist line circumference (96 cm, and for women 86 cm in waist line (13). Zhu and associates pointed out that waist line better predicts development of the cardiovascular diseases than BMI in patients of both gender.

A group of authors from our surrounding area, also support the attitude that waist line extent is a good indicator of the visceral obesity, that can independently point out health risk (12). Those values that indicate sudden rise of the risk factors for cardiovascular diseases for men are 96 cm, and for women 86 cm in waist line (13).

Notification of either of the disorders in patients with established disrupted AST/ALT (ratio-aspartate aminotransferase alanine aminotransferase), ratio, obesity, especially morbid obesity (BMI>35), DM type 2 diagnosis, diagnosis of the metabolic syndrome, obstructive apnea, point out towards NAFLD (2). Body mass measurements (BMI index), waist line measurements (VC) waist-hip ratio (VHR), bio electrical impedance analysis (BIA) are widely recommended in patients with NFDL as diagnostic tools, for being comfortable and relatively at low cost (2). In raised suspicion for NAFLD ultrasonographic imaging is diagnostic method first to be used in liver steatosis detection, in order to avoid more invasive methods as liver biopsy (1, 2).

Existing dogma posits that liver biopsy is the most reliable approach for identifying the presence of steato-hepatitis and fibrosis in patients with NAFLD, but it is generally acknowledged that biopsy is limited by cost, sampling error, and procedure-related morbidity and mortality. So, liver imaging is the common test used
was noted. Anthropometric characteristics, and their frequency among the tested groups are shown on the Table 2. and 58.46 % tested subjects of the group with NAFLD had BMI ≥35, what according to the WHO guidelines fall in obesity of second degree. Waist circumference > 88 cm had 88.64 % of the responders. These was followed by increased fat deposition, fat body value were detected in 32 % responders, between 20 and 25, while values >25, had 64.02 % of them. Responders with NAFLD had a significantly higher incidence of disorders compared with those in controls.

Comparing participants to the input of alcohol abuse and the factor of risk for the cardiovascular disease presence, we came out with results pointing on study group, which had significantly higher incidence of hypertension, diabetes, and hypercholesteremia (p<0.001), comparing with controls, also significantly higher incidence for obesity and diabetes was noted among close relatives (Table 3).

Fasting glucose and fasting insulin values, insulin resistance (HOMA-IR) were significantly higher in responders with NAFLD, also significantly positive correlation with BMI and waist circumference (Tables 4 and 5) established. Correlation between WHR relationship and insulin resistance was not confirmed (r =0.08782; p=0.3167).

4. DISCUSSION

There is all the more evidence that NAFLD presents hepatic component of the “metabolic syndrome” defined with obesity, hypercholesteremia, peripheral insulin resistance, diabetes mellitus, hypertriglyceridermia and hypertension (5,6,26,27). There are not that many studies that dwelt with anthropometric measurements in subjects with non-alcoholic fatty liver so assessed data are conflicting. Obesity is well documented factor of risk for NAFLD. Excessive obesity defined as BMI ≥25 and visceral obesity, waist circumference of ≥88 cm in women and ≥102 cm in men have been recognized as factors of risk for NAFLD (21). Our results confirmed that responders in study group were more obese (BMI 32.22±5.50kg/m² vs 24.67±3.83 ± 3, 51 kg/m²) than responders in controls which were, statistically highly significant (p <0, 001). In group of steatosis also were assessed statistically higher values for waist circumference 122.6±11.7 vs 86.0±5.8 cm (p<0, 001). Comparing data from available literature, studies from the region confirm facts that subjects with liver steatosis are significantly more obese than responders without steatosis (26, 27). The impact of the excessive body mass on liver disorders appearance confirmed studies that based diagnosis on liver biopsy. Ratziu V and associates confirmed in their studies, that obesity is the factor of risk in development of severe fibrosis (28). Cheung O and a group of authors based on collected data, that the degree of abdominal obesity correlates with necroinflamatory liver activity (29). Uslusoy HS and associates on the contrary did not establish statistically significant correlation between body mass index, waist circumference and severe necroinflamatory inflammation, respectively fibrosis (30).

Obesity is an independent risk factor for DMT2 de-

| Parameters                      | Study group (N130) | Control group (N40) | P-value |
|---------------------------------|-------------------|---------------------|---------|
| No of men                       | 78                | 24                  | 0.002   |
| Age (y)                         | 56.26             | 56.40               | 0.001   |
| BMI (kg/m2)                     | 32.22±5.50        | 24.67±3.83          | 0.001   |
| Waist circumference (cm)        | 122.6±11.7        | 86.0±5.8            | 0.001   |
| Waist-to-hip ratio (WHR)        | 0.96±0.70         | 0.88±0.12           | 0.502   |
| Total cholesterol (mmol/L)      | 6.47±1.17         | 5.34±0.80           | 0.001   |
| HDL-cholesterol (mmol/L)        | 1.34±0.44         | 1.49±0.46           | 0.06    |
| LDL-cholesterol (mmol/L)        | 4.30±0.92         | 3.20±0.79           | 0.001   |
| Tryglicerides (mmol/L)          | 2.77±1.10         | 1.58±1.58           | 0.001   |
| Fasting blood glucose (mmol/L)  | 6.02±0.95         | 4.88±0.46           | 0.001   |
| Blood pressure (mm Hg)          |                   |                     |         |
| Systolic                        | 131.7±15.1        | 119.8±14.4          | 0.001   |
| Diastolic                       | 87.7±10.0         | 78.9±9.0            | 0.001   |
| Fasting blood insulin (ml/L)    | 15.7 ± 7.6        | 6.00 ± 2.8          | 0.01    |
| HOMA-IR index                   | 3.9 ± 2.8         | 1.2 ± 0.6           | 0.001   |
| CRP                             | 6.7±4.88          | 5.83±6.47           | 0.037   |
| Fibrinogen (g/L)                | 3.67±2.54         | 3.33±0.95           | 0.45    |
| AST(U/L)                        | 65.01±12.8        | 29.45±9.8           | 0.97    |
| ALT(U/L)                        | 52.27±12.8        | 22.33±12.8          | 0.004   |
| AST/ALT ratio > 1%              | 100               | 2                   | 2.001   |

| Parameters                      | Study group (N130) | Control group (N40) | P-values |
|---------------------------------|-------------------|---------------------|----------|
| Alcohol intake (g/d)            | 12.8              | 13.00               | 0.245    |
| Glycemic load                   | 63.85             | 7.50                | 0.0001   |
| Physical activity (MET)         | 11.2              | 15.30               | 0.239    |
| Current smoker (%)              | 33.08             | 45.00               | 0.2355   |
| Family history of obesity (%)   | 58.46             | 12.71               | 0.001    |
| Family history of diabetes (%)  | 27.69             | 5.05                | 0.001    |
| Hypertension (%)                | 43.08             | 17.50               | 0.001    |
| High cholesterol (%)            | 46.15             | 7.50                | 0.001    |

Table 1. The values of assessed anthropometric, clinical and laboratory data (expressed as X±SD) of patients with NAFLD and control group

Table 2. Anthropometric caracteristics, body fat and their frequency in tested groups.

Table 3. Comparaison of the participants to the input of alcohol abuse and the factor of risk for the cardiovascular deaseses presence.
Assessed as HOMA-IR index > 3, established in the group with NAFLD, with average 3.9 ± 2.8 vs 1.2 ±0.6 values, which was statistically significant. Results very similar to ours obtained Ana Lúcia Farias de Azevedo Salgado (32). In the preceding study biochemical analysis of the 116 patients with NAFLD biopsy verified diagnosis, were compared with the results of the healthy subjects. NAFLD patients had higher levels of insulin, glycemia and HOMA-IR values, compared to controls, even when they did not have diabetes or glucose intolerance.

In addition to related disorders of glucose metabolism and insulin resistance, we found a large frequency of the hypertension in patients with NAFLD. Lopez-Suarez and ass (33) presented in their observation study, that percentage of the participants with hypertension were 21, 2% higher comparing to those without NAFLD (95% CI, 11.8–30.6. P < 0.0005). Numerous studies of other authors do not justify the importance of elevated values of the liver enzymes and their impact for the diagnosis of nonalcoholic fatty liver, one of those is study conducted by Noguchi and ass (34), while several cross sectional studies found firm connection with elevated ALT, γ-GT values, and metabolic syndrome, diabetes and NAFLD (35, 36, 37, 38, 39). Investigating the importance of the liver enzymes disorders Goessling and assoc. state that in general population high aminotransferase (a surrogate marker for NAFLD) increase long term risk for diabetes, metabolic syndrome and cardiovascular incidents (36). Degree of enzyme is not determined, it is usually between 1 and 4 times over upper limit. Although amino- transferase (ALT) values are higher than aspartate aminotransferase (AST) levels, in most cases (37) the AST levels temporarily could be higher than levels of AST, especially in cirrhosis (38). All the same, the fact is that AST / ALT ratio never exceeds 2.

Kotronen and associates in their research pointed out that AST and ALT values were higher in patients with DM type 2, from 40 up to 200 % comparing to those without diabetes and fatty liver (39). Also studies with conducted by Duvnjak et al. Most of the patients had ALT levels higher than AST, although with the development of fibrosis, AST could be found significantly increased (40). One of the requirements for inclusion in this study was disrupted AST/ALT ratio. Data from our study implicate higher γ-GT values, statistically high significant in patients with NAFLD. New American guidelines, unfortunately does not support non invasive biomarkers for identification and prediction NAFLD (1) despite great interest and ongoing numerous studies.

Finally, by analyzing the above mentioned facts, it can be observed that despite the presence of disturbed anthropometric parameters, responders with non alcoholic fatty liver have also disrupted specific biochemical findings. Presented data confirmed significant deviation from the normal values in levels of glucose, liver enzymes, γ GT, insulin but lipids too. Patients with NAFLD have disrupted lipid status, concluded by several studies (41, 42, 43) as for our results they are in accordance to the cited studies. Accumulation of triglyceride in hepatocytes is considered the main pathogenic trigger in the development of NAFLD (44). Cholesterol metabolism in patients with NA-

| Parameters | Correlation |
|------------|-------------|
| Glycemia (mmol/L) r = 0.1322; p=0.07 |
| Triglycerides (mmol/L) r = -0.06499; p=0.4591 |
| Total cholesterol (mmol/L) r = -0.0922; p=0.2929 |
| γ GT(U/L) r = 0.1567;p=0.0727 |
| Insulinemia (mIU/L) r=0.2028; p=0.01 |
| HOMA-IR r=0.151; p<0.05 |

Table 4. Correlation between BMI, glucose values, triglycerides, total cholesterol values, insulinemia and HOMA-IR index. Pearson’s correlation coefficient p-level of the correlation significance

| Parameters | Correlation |
|------------|-------------|
| Glycemia (mmol/L) r=0.2265; p<0.01 |
| Triglycerides (mmol/L) r= -0.1085; p =0.2154 |
| Total cholesterol (mmol/L) r=-0.04339; p=0.6213 |
| γ GT(U/L) r=0.1567;p<0.05 |
| Insulinemia (mIU/L) r=0.2981; p<0.01 |
| HOMA-IR r=0.1502; p=0.0858 |

Table 5. Correlation between waist circumference, glucose values, triglycerides, total cholesterol values, insulinemia and HOMA-IR index. Pearson’s correlation coefficient p-level of the correlation significance

| Parameters | Correlation |
|------------|-------------|
| Glycemia (mmol/L) r=-0.1751; p<0.05 |
| Tryglycerides (mmol/L) r= 0.02179; p =0.121 |
| Total cholesterol (mmol/L) r=-0.07594;p=0.3868 |
| γ GT(U/L) r=0.1567;p<0.05 |
| Insulinemia (mIU/L) r=-0.2790; p<0.05 |
| HOMA-IR r=-0.08782; p=0.3167 |

Table 6. Correlation between WHR, glucose values, triglycerides, total cholesterol values, insulinemia and HOMA-IR index. Pearson’s correlation coefficient p-level of the correlation significance
FLD is still insufficiently explored, but it is well known fact that insulin resistance is associated with increased cholesterol synthesis (45). In these patients in order to maintain lipid homeostasis, synthesis of VLDL (very-low density lipoproteins) increases, increased levels of VLDL leads to rise of LDL cholesterol levels (46). The results of our study go along with the last statement in the line, raised levels of the cholesterol of LDL cholesterol was established in patients with NAFLD (47). In order to assess the size of the total fat mass, we used reference values by Bray, so referent values FAT% for men were 12-20%, and for women were 20-30%. This method confirmed that participants with nonalcoholic fatty liver have significantly greater amount of the fat mass, than the controls.

5. CONCLUSION
Our results revile that patients with nonalcoholic fatty liver excessively obese, have enlarged waist circumference, consequential insulin resistance, and impaired glucose metabolism, impaired lipid profile, and thus the risk of developing cardiovascular diseases.

CONFLICT OF INTEREST: NONE DECLARED

REFERENCES
1. Chalasañi N, Younossi Z, Lavine JE, Diel PB, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012 Jun; 55(6): 2005-2023.
2. World Gastroenterology Organisation Global Guidelines, Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis, June 2012.
3. Boza C, Riquelme A, Ibañez L, Duarte I, Norero E, Viviani P, Soza A, Fernandez JJ, Raddatz A, Guzman SA. M. Predictors of non- alcoholic steatohepatitis (NAS) in obese patients undergoing gastric bypass. Obes Surg. 2005; 15: 1148-1153.
4. Vernon G, Baranowa A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011; 34: 274-285.
5. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002; 346: 1221-1231.
6. Nyschewander Tieti RA, Caldwell SH. Nonalcoholic Steatohepatitis: Summary of an AASLD Single Topic Conference. Hepatology 2003; 37: 1202-1219.
7. Kotronen A, Yki-Järvinen H, Männistö S, Saarikoski L, Korpi-Hyövälti E, Oksa H, Saltevo J, et al. Non-alcoholic and alcoholic fatty liver disease—two diseases of affluence associated with the metabolic syndrome and type 2 diabetes: the FIN-D2D survey. BMC Public Health. 2010; 10: 237.
8. Novaković D. Ultrazvučni nalazi jetre radnika u tekstilnoj industriji za postavljanje dijagnoze uslovljenih metabolizma. SPSZ arhiv za celokupno lekarstvo. 2007; 9: 526-535.
9. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibaud V, Theodorou I, et al. Liver fibrosis in overweight patients. Gastroenterology. 2000, 118(6): 1117-1123.
10. Cheung O, Kapoor A, Puri P, Sistrun S, Luketic VA, Sargeant CC, et al. The impact of fat distribution on the severity of non-alcoholic fatty liver disease and metabolic syndrome. Gastro. 2000; 147: 165-169.
11. Reaven GM. Banting lecture 1988: role of insulin resistance in human disease. Diabetes. 1988; 37: 1595–607.