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Elevated serum Homocysteine levels a possible non-invasive diagnostic biomarker in patients with Non-alcoholic fatty liver disease

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Cover Page Footnote

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ORIGINAL STUDY

Elevated Serum Homocysteine Levels a Possible Non-invasive Diagnostic Biomarker in Patients With Non-alcoholic Fatty Liver Disease

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Abstract

Lack of independent biomarkers is very much evident in NAFLD. Early detection of NAFLD is difficult due to the absence of specific diagnostic and prognostic markers and clinical symptoms. We retrospectively collected the information of patients hospitalised with NAFLD diagnosis and metabolic syndrome during 2019−2020 using the tertiary care hospital inpatient sample database and evaluated the changes in their serum homocysteine levels. We found that 59.063% of NAFLD in the male population and 41.667% of NAFLD in the female population had increased serum homocysteine. This shows that elevated serum homocysteine can act as a potential biomarker for NAFLD.

Keywords: Fatty liver, Homocysteine, Non-alcoholic steatohepatitis, Retrospective analysis, Obesity, Biomarker

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a major and serious health issue in both the western and eastern hemispheres of the globe and a paramount health problem in the Indian sub-continent [1,2]. The important hallmarks of NAFLD progression include simple steatosis (simple fatty liver), nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis [3]. Though early diagnosis of NAFLD supplemented with lifestyle modification and calorie restriction can reverse the condition "in the initial stages of NAFLD", poor lifestyle and delayed diagnosis advance the disease progression leading to loss of liver function due to scarring and accumulation of excessive collagen fibres in the liver [4]. Several hallmarks of metabolic syndrome like obesity, diabetes, hyperlipidaemia, and cardiovascular disease (CVD) are also directly associated with NAFLD [5]. Long-term progression of NAFLD causes cirrhosis and hepatocellular carcinoma (HCC), and can ultimately result in terminal liver failure [6]. So, early detection of NAFLD will help in preventing the disease progression.

Recent studies have shown that serum homocysteine might be a major risk factor in NAFLD and thus has all the features of a potential biomarker [7,8]. A recent report even points to a direct link between dietary supplements such as vitamins mainly folic acid, vitamin B12, and homocysteine levels [9]. Metabolically homocysteine is a product of methionine, a sulphur-containing amino acid, and its levels are significantly higher in patients with comorbidities such as obesity, type 2 diabetes, and CVD [10]. Some studies have also pointed toward an increased homocysteine level in alcoholic liver damage [11,12].

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The present study aims to differentiate the levels of serum homocysteine in NAFLD individuals. We performed a retrospective analysis to determine whether there are any elevated homocysteine levels in NAFLD subjects in the Indian population and compared the serum homocysteine levels of male and female subjects included in our retrospective study.

2. Subjects and methods

A retrospective study was performed on 107 NAFLD individuals (83 males, 24 females) aged between 18 and 60 years old, who underwent routine medical check-ups in the tertiary care hospital, Mysore, India between January 2019 and December 2020. All the subjects recruited for our study had NAFLD. We collected the serum homocysteine levels data and compared them between male and female subjects. Serum homocysteine levels of $\leq$5 μmol/L were considered as low and $\geq$20 μmol/L were considered as high and a range of values between 5 and 20 μmol/L was considered normal.

2.1. Exclusion criteria

We had total 620 male subjects and 360 female subjects (Jan 2019-Decmber 2020). Some of these patients had stroke, viral hepatitis, pneumonia, seizure, hypothyroidism, anaemia, hepatomegaly, CVDs and type 2 diabetes (Table 2). We excluded all of them while retaining only confirmed NAFLD subjects. Therefore, finally we arrived at 107 subjects.

2.2. Homocysteine estimation

Serum Homocysteine levels were measured by enzymatic method using COBAS-6000 fully automated Chemistry Analyzer (Reference Range: Male: 6–22 μmol/L, Female: 3–18 μmol/L).

3. Results

Based on our study we had a total of 83 male NAFLD subjects and 24 female subjects. Out of 83 male subjects, 49 had high homocysteine and 33 had normal homocysteine and one subject had a very low homocysteine level. Out of 24 female subjects, 14 had normal homocysteine and ten had high homocysteine (Table 1). Some of these subjects also had cardiovascular disease, Type 2 diabetes, hypertension, hypothyroidism, and hepatomegaly. Males exhibited increased Homocysteine levels prevalence of homocysteine compared with females (59.036% vs 41.667%) (Fig. 1) (see Table 3).

4. Discussion

It is very well known that the incidence of NAFLD increases with a sedentary lifestyle, high-calorie food intake, and obesity. Previously the development of NAFLD was very uncommon in children.

Table 1. Table showing the total number of male and female subjects with NAFLD and the corresponding low, normal, and high serum homocysteine levels respectively involved in the study.

Particulars	Male				Female			
	Total no. of cases	Low HCY	Normal HCY	High HCY	Total no. of cases	Low HCY	Normal HCY	High HCY
Fatty liver	83	01	33	49	24	0	14	10

Table 2. Table showing the total number of male and female subjects and their corresponding low, normal, and high serum homocysteine levels respectively involved in the study along with lipid profile and liver enzymes (Including which are excluded based on exclusion criteria).

Contents	Male (Total = 620)				Female (Total = 360)			
	No data	Low	Normal	High	No data	Low	Normal	High
HCY	–	**29**	**297**	**294**	–	**16**	**265**	**79**
Random Blood Sugar	133	2	330	155	59	3	244	53
Fasting Blood Sugar	503	3	37	77	304	0	27	28
Post-Prandial Blood Sugar	534	1	25	60	329	0	16	14
HIgh-Density Lipoprotein	355	256	13	–	250	108	1	–
Low-Density Lipoprotein	259	–	159	202	217	–	60	82
Cholesterol	259	–	291	70	216	–	109	34
TG	258	–	211	151	216	–	100	43
Alkaline Phosphatase	243	1	190	186	111	1	63	184
Alanine Transaminase (SGPT)	240	–	280	100	106	–	215	38
Aspartate Transaminase (SGOT)	240	–	269	111	106	–	192	61

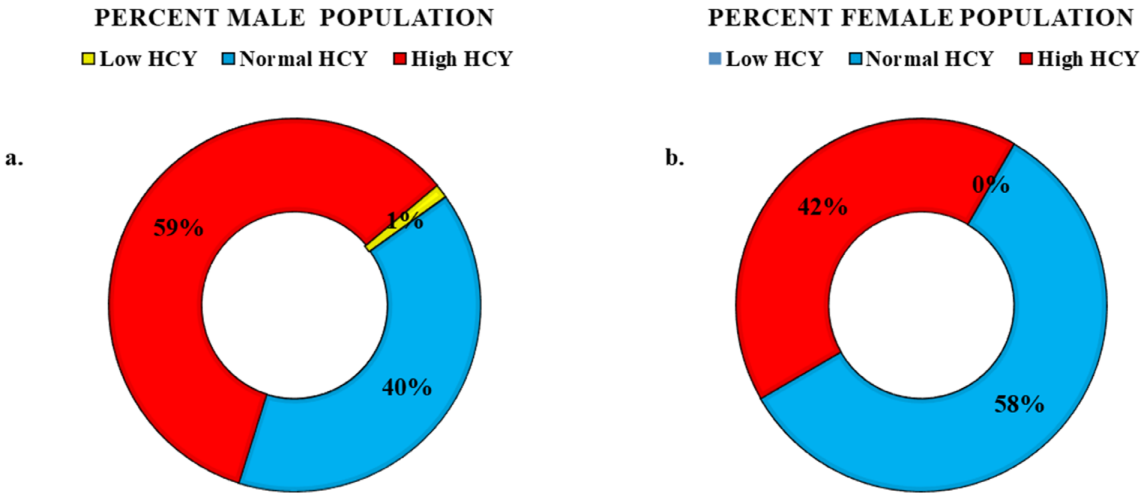

Fig. 1. Graphical representation of the percentage of a. Male and b. Female NAFLD patients with low, normal, and high serum homocysteine levels. About 59% of the male and 42% of the female NAFLD patients showed a correlation with high serum homocysteine levels.

Table 3. Table showing the total number of male and female subjects with different metabolic parameters and the corresponding low, normal, and high serum homocysteine levels respectively involved in the study (Including which are excluded based on exclusion criteria).

Contents	Male				Female			
	Total no of cases	Low HCY	Normal HCY	High HCY	Total no of cases	Low HCY	Normal HCY	High HCY
Stroke	94	5	35	54	35	2	27	6
CVT	62	1	19	42	37	6	21	10
DVT	75	3	33	39	16	2	12	2
CVA	124	5	66	53	37	3	24	10
MCA	22	0	11	11	5	0	3	2
T2DM	86	4	58	24	26	6	16	4
Hypertension	116	4	62	50	40	7	30	3
Anemia	16	1	11	4	23	3	15	5
B12 Deficiency	5	0	4	1	3	0	3	0
Splenomegaly	14	1	7	6	4	0	3	1
Fatty liver	83	1	33	49	24	0	14	10
CVD'S	45	0	18	27	17	2	12	3
PVT	15	1	9	5	9	1	8	0
Hypothyroidism	4	0	2	2	17	1	14	2
Hepatitis	3	0	2	1	1	0	1	0
Seizure	20	2	16	2	15	0	13	2
Pneumonia	13	0	11	2	5	0	3	2
Hepatomegaly	15	1	9	5	6	0	5	1

CVT, Cerebral Venal Thrombosis; DVT, Deep Vein thrombosis; CVA, Cerebrovascular Accident; MCA, Middle Cerebral Artery; T2DM, Type 2 Diabetes Mellitus; CVD'S, Cardiovascular Diseases; PVT, Portal Vein Thrombosis.

Globalisation and its consequences like lifestyle changes have made its occurrence very common in recent years [13]. The occurrence of NAFLD is said to follow a pattern with age and sex (with men at a higher risk than women) being the common determinants, both in developing and developed countries [1]. The data criteria and similarity distribution of patients with NAFLD in our study mimic are almost similar to the past studies conducted [14–16]. Our study has further differentiated the serum homocysteine levels in male and female populations with the preponderance in male subjects being more than that of female subjects. In our study, NAFLD was found in middle-aged (25–55yrs) as compared to T2DM-related NAFLD.

Based on our retrospective analysis, we found that 59.036% of male NAFLD subjects correlated with increased serum homocysteine. Such a correlation was not observed in the female population included, pointing towards a sex-specific association between serum homocysteine level and NAFLD. Our results suggest that serum homocysteine levels might be positively associated with the occurrence and progression of NAFLD in men but not in women and

are in turn strongly supported by an elegant study involving the Korean population [17]. Radiological diagnosis of the NAFLD development and progression, followed by the detection of serum homocysteine will help us validate the possibility of NAFLD progression in the male population.

The results from our study also correlated with that of a few studies reported in India and other countries [18,19]. The biochemical, clinical, and pathological symptoms of NAFLD patients in our study showed a strong positive correlation with stroke, hypertension, T2DM, and CVDs as well as overall metabolic syndrome. The incidence of hyperglycemia and hypertension with NAFLD were the commonly reported comorbidities in our study subjects. The advanced stages of NASH were reported only in two patients among the subjects involved in the study.

Based on our study and other previous reports, we suggest that there is an immediate need to design a better screening programme for patients with metabolic syndrome and NAFLD, and a critical need to look for liver injury, inflammation, fibrosis, and other NAFLD markers validated by biochemical, and immunological, pathological and cytohistological methods supplemented with advanced machine learning and artificial intelligence methods. Though our study highlights the sex-specific disparity observed in the homocysteine levels in males than in females, it is to be noted that quantitatively the female population considered is comparatively lesser than the total male subjects with NAFLD (24 vs 83). This highlights the urgent need to design studies based on larger cohorts and study populations to validate the findings of studies involving smaller target populations and to confirm the elevated serum Homocysteine levels as a bonafide non-invasive diagnostic biomarker in patients with NAFLD.

Conflict of interest

We don't have any conflict of interest to disclose. Since the data was obtained from the tertiary care hospital patient repository, prior permission was obtained from the institutional review committee.

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