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To Study the Risk of Hepatocellular Cancer in Patients with Non-Alcoholic Fatty Liver Disease in Central India

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***Abstract*---**Background: Hepatocellular carcinoma (HCC) is the world's fifth most common cancer and the third leading cause of cancer-related deaths. A known risk factor for HCC is non-alcoholic fatty liver disease (NAFLD), a continuum of hepatic disorders related to obesity and the metabolic syndrome. Aim: We conducted an observational study to identify risk factors for hepatocellular cancer in patients with non-alcoholic fatty liver disease who came to SMHRC Nagpur for a routine visit. Material and Methods: The study included 300 people aged 35 to 85 years old who visited Shalinitai Meghe hospital in Nagpur for a health check-up. We were able to keep the two groups apart here. The control group is made up of alcoholics with fatty liver, while the study group is made up of non-alcoholics with fatty liver. Each community consists of 150 patients. A quantitative diagnostic kit was used to analyse liver function and lipid profile analyses, which were then examined using a photometric process. The enzyme related immunosorbant assay was used to detect glutathione s transferase pi. RESULTS: Non-alcoholic fatty liver had

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greater LFT than alcoholic fatty liver in the control group, according to the report.

***Keywords*---**GST-π, HCC, hepatitis, liver cancer, NAFLD, outcome.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide, with a pathological spectrum that includes anything from basic steatosis [non-alcoholic fatty liver (NAFL)] to varying degrees of inflammation and liver cell injury, a disorder known as non-alcoholic steatohepatitis (NASH) (1, 2). The current rise in the prevalence of NAFLD and NASH may be attributed to associated rises in obesity and metabolic syndrome (3, 4, 5). Hepatocellular carcinoma (HCC) is the world's fifth most common cancer, accounting for around 5% of all malignant tumors in humans.[6] Many nations, including America, Europe, and Asia, have seen an increase in incidence in recent years (7, 8). Idiopathic HCC accounts for 15–50% of HCC cases, implying that other risk factors are to blame for the disease's rising prevalence. In 5–30% of patients with advanced liver disease, cryptogenic cirrhosis (CC) is found. Recent research indicates that these "idiopathic" cases may be related to nonalcoholic fatty liver disease (NAFLD) (9). NAFLD is a slow-progressing condition that can lead to cirrhosis and liver failure. In the last few years, NAFLD has received more attention because of its high prevalence worldwide (10).

In the United States, the age-adjusted incidence of HCC has increased from 1.5 to 4.9 per 100,000 people in the last 30 years (11). Around 782,000 new cases of liver cancer were diagnosed in 2012, with 746,000 deaths worldwide (12). Wide population-based surveys across continents have recorded proportions of NAFLD HCC ranging from 14.1% (13) to 21.5 % (14) with an annual increase in NAFLD HCC of 9% in the US (13), a more than 10-fold increase in the UK (14) between 2000 and 2010, and an increase in Korea from 3.8 % in 2001–2005 to 12.2 % in 2006–2010.15 (15). NASH is responsible for a large portion of HCC despite accounting for a limited portion of NAFLD. The prevalence of NASH HCC varies by geography, ranging from 10–12 % in North America and Europe to 1–6% in Asia[16]. It appears to be the source of HCC that is spreading the fastest (13).

Differential development of these liver diseases may be influenced by both endogenous and exogenous influences (17, 18). Environmental elements that contribute to oxidative stress, for example, can exacerbate hepatic injury in certain patients, while genetic factors can play a larger role in others (19, 20). Furthermore, a combination of these factors may have an effect on the disease's progression by tipping the scales in favor of progression. In this sense, enzymes including glutathione-S-transferases (GST), which are responsible for the detoxification of potentially harmful by-products of ethanol metabolism like acetaldehyde and reactive oxygen species, (21) have been identified as possible candidates for NAFLD susceptibility. Given this ambiguity, the primary goal of our research is to investigate the relationship between the LFT, lipid profile, and GST, as well as NAFLD susceptibility.

Material and Methods

Our research was carried out in the Biochemistry Department of DMMC&SMHRC Nagpur from October 2020 to March 2021. A total of 300 subjects aged 35 to 85 years old were enrolled in this study. There were 150 Non alcoholic with fatty liver in the study group and 150 non alcoholic in the control group out of a total of 300 participants. For this study, 300 patients between the ages of 35 to 85, both sexes, who Non alcoholic and suffered from fatty liver disease and came to Shalinitai Meghe Hospital Nagpur for their regular check up was chosen.

Inclusion criteria

- This study includes the Non alcoholic persons those suffer from the fatty liver disease and also include alcoholic persons with fatty liver in this study.
- Informed consent was taken from all participants prior to the study.

Exclusion criteria

- The study included no underweight participants, pregnant women, individuals with malignancies/infections.
- Eligible candidates not willing to participate.

Blood sample collection and processing

Non fasting blood sample was obtained in a vacutainer containing gel clot activator from the median cubital vein with tourniquet attached to the limb and fingers squeezed. Blood was centrifuged for 10 min at 10,000 rpm to settle all the formed elements and separate serum. Samples were analyzed in the clinical chemistry laboratory of Shalinitai Meghe Hospital Nagpur. Glutathione s transferase-π lipid profile and liver function test were measured within 24 h from samples obtained from the patients. Aliquots of the samples were frozen at -80^{0}C for subsequent assessment of glutathione isoenzyme-π, lipid profile and liver function test.

Biochemical analysis

Hepatic enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as the lipid profile like triglyceride (TG), total cholesterol (TC), were tested using a quantitative diagnostic kit and a photometric process. An enzyme-linked immunosorbant assay was used to confirm the presence of GST pi in heparinized whole blood.

Definitions of alcohol intake

Based on published reports, the degree of alcohol consumption was divided into four groups (22). The first group was “never," which was described as abstaining from all alcohol consumption. The second category was referred to as "social alcohol intake," which was described as no more than two alcoholic drinks per day on weekdays and three to six drinks per day on weekends. Significant alcohol consumption was described as more than two drinks per day or more than six

drinks per day on weekends for the previous five years. More than “social alcohol intake" within the previous 5 years was described as “formerly significant alcohol intake."

Statistical analysis

Alcoholic and Non-alcoholic with fatty liver groups, as well as GST-π-deficient and non-deficient groups were compared using the Student t test and the x^2 test. Data for all parameters was evaluated for means and standard deviation. Data processing was carried out by Microsoft Excel and the social sciences statistical kit (SPSS version 22).

Result

Table 1
Characteristics of alcoholics and non-alcoholics, lipid profile, and liver function tests

Sr.No	Characteristics	Non- alcoholic with fatty liver (N= 150)	Alcoholic with fatty liver (N=150)
1.	Age	55.5±10.9	45.6± 12.3
2.	Body mass index	62.5±15.3	50.4±9.1
3.	ALT	70.8±13.2	60.3±10.8
4.	AST	75.4±18.5	65.1±11.7
5.	Billirubin	49.2±7.8	40.1±5.7
6.	ALP	85.6±16.4	73.9±12.8
7.	Albumin	51.2±10.5	42.6±7.4
8.	Serum cholesterol	115.4±35.2	80.1±20.4
9.	Serum triglyceride	148.7±40.6	73.8±17.9

Abbreviations

BMI stands for body mass index; ALT stands for alanine aminotransferase; AST stands for aspartate aminotransferase.

Table 1 displays the data for the non-alcoholic and alcoholics with fatty liver categories. There is no difference between alcoholics and non-alcoholics in terms of age or BMI in that table. In this table, non-alcoholic with fatty liver had substantially higher levels of all liver function test parameters, as well as statistically higher levels of total cholesterol and triglyceride, when compared to alcoholic with fatty liver. (The research community vs. the monitoring group)

Table 2
Alcoholics and non-alcoholics with fatty livers have different GST status.

Sr.No	Alcoholic and Non alcoholic status	GST-πPositive	GST-π Negative
1	Non alcoholics with fatty liver (N=150)	125.7±36.4	95.7±13.8
2	Social alcohol intake (N=50)	65.3±8.9	68.9±9.9

3	Significant alcohol intake (N=50)	50.7±10.2	59.7±12.4
4	Formerly significant alcohol intake (N=50)	42.1±5.6	54.6±6.7

Alcoholics have three common alcoholic statuses, as seen in Table 2. In contrast to alcoholics with fatty liver, GST- was found to be mostly positive in non-alcoholics with fatty liver. In comparison to the non-alcoholic population, GST- is negative in social alcohol consumption, substantial alcohol intake, and previously significant alcohol status. As a result, the GST pi was elevated in all of this alcoholic population, but not significantly higher than in non-alcoholics with liver disease. GST-pi has a higher social alcohol intake than the other two alcoholic categories.

Discussion

In India, the rise in HCC incidence has largely coincided with the obesity epidemic. New evidence suggests that NAFLD is a key factor in the association between obesity and HCC (24). Nearly two-thirds of obese people are thought to have some type of fatty liver, ranging from steatosis to NASH. NASH can lead to liver cirrhosis in 3 percent to 15% of people (24) and, in the worst-case scenario, liver cancer. Glutathione-S-transferases enzymes can detoxify harmful ethanol metabolites in the liver by conjugating acetaldehyde and reactive oxygen species (ROS) to reduced glutathione, which is an essential protection against cellular damage caused by chemical and oxidative stress (25). There is no important association between age and NAFLD, according to Pardhe et al.2018 (26). The mean levels of hepatic enzymes were higher in the NAFLD community in this analysis, and apart from ALP, there was a significant association with NAFLD in other cases. Novakovic and colleagues (Novakovic et al.2014) (27). Apart from AST and NAFLD, an important relationship was found between hepatic enzymes in the sample. The majority of previous research has found a connection between NAFLD and AST, ALT, and ALP levels. According to Zakeri and Karmarat-Panah 2018 (29), ALT and dyslipidemia may play a role in the prevalence and progression of NAFLD.

In Nepal, Pardhe et al. 2018 discovered a correlation between hepatic enzymes (ALT, ALP) and dyslipidemia, as well as different grades of NAFLD. Santhosha kumari et al. 2017[28] found that patients with NAFLD had higher TC, LDL, and TG, as well as lower HDL, when compared to the control group, and that dyslipidemia was substantially higher in the NAFLD group. Furthermore, Novakovic et al. 2014 found an important relationship between TG, LDL, TC, and HDL, as well as an inverse relationship with HDL, when comparing chemical parameters with NAFLD in Serbia. They found similar findings in studies by Pardhe et al. 2018[26] and Jain et al. 2018. Harada et al. 1987 found that liver biopsy samples from a Japanese population of patients with hepatitis and carcinoma were more likely to produce pi class glutathione S-transferase in non alcoholic fatty liver disease. However, sinusoidal macrophages, Kupffer cells, and hepatocytes express GST-pi in non-alcoholic liver disease, according to Harrison DJ 1990[32]. Howie AF1989[33] There's also evidence that biliary epithelial cells secrete GST-pi into bile as a way to get rid of potentially dangerous toxins.

Conclusion

The findings of this study revealed that biochemical markers in patients with NAFLD have changed significantly. As a result, it appears that in clinical settings where biochemical and lipid changes are detected, sonography should be used to test individuals with NAFLD, as early diagnosis avoids and delays further complications. The increased GST enzymatic activity, on the other hand, indicates that patients with NAFLD have a restricted ability to handle electrophilic compounds. The high incidence of NAFLD is worrying for population health as well as opioid treatment regimens.

References

1. Stine JG, Wentworth BJ, Zimmet A, Rinella ME, Loomba R, et al. Systematic review with meta-analysis: risk of hepatocellular carcinoma in non-alcoholic steatohepatitis without cirrhosis compared to other liver diseases. Aliment Pharmacol Ther 2018;48:696- 703
2. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388-402.
3. Falck-Ytter Y, Younossi ZM, Marchesini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. Semin Liver Dis 2001;21:17-26.
4. Younossi ZM, Diehl AM, Ong JP. Nonalcoholic fatty liver disease: an agenda for clinical research. HEPATOLOGY 2002;35:746-752.
5. Reid AE. Nonalcoholic steatohepatitis. Gastroenterology 2001;121:710- 723.
6. D. M. Parkin, F. Bray, J. Ferlay, and P. Pisani, “Estimating the world cancer burden: Globocan 2000,” International Journal of Cancer, 2001; 94(2), 153–156.
7. J. Bruix and M. Sherman, “Management of hepatocellular carcinoma,” Hepatology, 2005;42(5), 1208–1236.
8. H. B. El-Serag, “Hepatocellular carcinoma: recent trends in the United States,” Gastroenterology, 2004,127(5), 27–34.
9. E. Bugianesi, N. Leone, E. Vanni et al., “Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma,” Gastroenterology, 2002
10. ; 123(1), 134–140.
11. A.J. McCullough, “The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease,” Clinics in Liver Disease, 2004;8(3), 521–533.
12. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol 2009;27:1485-91.
13. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
14. Younossi ZM, Otgonsuren M, Henry L, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. Hepatology 2015;62:1723-30

15. Dyson J, Jaques B, Chattopadyhay D, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. J Hepatol 2014;60:110-7.
16. Cho EJ, Kwack MS, Jang ES, et al. Relative etiological role of prior hepatitis B virus infection and nonalcoholic fatty liver disease in the development of non-B non-C hepatocellular carcinoma in a hepatitis B-endemic area. Digestion 2011;84 (1):17-22
17. Park JW, Chen M, Colombo M, et al. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. Liver Int 2015;35:2155-66
18. Day CP, James OFW. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842-845
19. Fong DG, Nehra V, Lindor KD, Buchman AL. Metabolic and nutritional considerations in nonalcoholic fatty liver. HEPATOLOGY 2000;32:3-10.
20. Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. JAMA 1999;282:1659-1664.
21. Rashid A, Wu TC, Huang CC, Chen CH, Lin HZ, Yang SQ, et al. Mitochondrial proteins that regulate apoptosis and necrosis are induced in mouse fatty liver. HEPATOLOGY 1999;29:1131-1138.
22. Boyer TD. The glutathione S-transferases: an update. Hepatology 1989; 9: 486–96.
23. Metwally MA, Zein CO, Zein NN. Predictors and noninvasive identification of severe liver fibrosis in patients with chronic hepatitis C. Dig Dis Sci 2007;52:582-588
24. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123:134-140
25. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346: 1221-1231.
26. Hirano T, Kaplowitz N, Tsukamoto H, Kamimura S, Fernandez-Checa JC. Hepatic mitochondrial glutathione depletion and progression of experimental alcoholic liver disease in rats. Hepatology 1992; 16: 1423–7.
27. Pardhe BD, Shakya S, Bhetwal A, Mathias J, Khanal PR, Pandit R, et al. Metabolic syndrome and biochemical changes among non-alcoholic fatty liver disease patients attending a tertiary care hospital of Nepal. BMC Gastroenterol. 2018;18:109.
28. Novakovic T, Mekic M, Smilic L, Smilic T, Inić-Kostic B, Jovicevic L, et al. Anthropometric and biochemical characteristics of patients with nonalcoholic fatty liver diagnosed by non-invasive diagnostic methods. Med Arch. 2014;68:22–6.
29. Santhoshakumari TMJ, Radhika G, Kanagavalli P. A study of anthropometric and lipid profile parameters in non-alcoholic fatty liver disease patients attending a tertiary care hospital at puducherry. IOSR J Dent Med Sci (IOSR-JDMS) 2017;16:33–7
30. Shinde R. A Prospective Observational Case Series of Liver Injury in Paediatric Patients Secondary to Consumption of Ayurvedic Herbomineral Formulations. Indian Journal of Forensic Medicine & Toxicology, October-December 2020, Vol. 14, No. 4; 7121-7125.

31. Ain P, Parate R, Dubey T, Jain R. Prevalence of NAFLD (non-alcoholic fatty liver disease) in metabolic syndrome and their correlation with various biochemical and serologic parameters for early detection and detecting patients of Non-alcoholic steatohepatitis Prevalence. 2018;3:24–8.
32. Harada S, Abei M, Tanaka N, Agarwal DP, Goedde HW. Liver glutathione S-transferase polymorphism in Japanese and its pharmacogenetic importance. Hum Genet 1987; 75: 322-5.
33. Harrison DJ, Hayes PC. Immunolocalisation of glutathione S-transferases in human renal and liver diseases. In: Hayes JD, Hayes PC, Mantle TJ, Pickett CB, eds. Glutathione S-transferase and drug resistance. London: Taylor and Francis, 1990: 431-41.
34. Howie AF, Forrester LM, Glancy MJ et al. Glutathione S-transferase and glutathione peroxidase in human tumors. Carcinogenesis 1990; 11: 451-8.