

ASSOCIATION OF INTESTINAL AMOEBIASIS WITH NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)- A CASE-CONTROL STUDY

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ABSTRACT

BACKGROUND

Little work has been done to show the association of intestinal amoebiasis with the nonalcoholic fatty liver disease. There are so many factors, which are related with the causation of NAFLD as for example obesity, diabetes mellitus, hypertension, hypercholesteraemia and sedentary living. Besides these, there are many unknown or little known factors, which should be studied. Amoebiasis is very common infection in North India including Gangetic plain of Bihar. These two conditions are very frequently observed together in a same person; therefore, a case-control study was done to confirm the association between these two conditions.

MATERIALS AND METHODS

In the present study, 120 cases of diagnosed NAFLD were taken of the age group between 30 years to 60 years, both male and female. 130 persons who were not suffering from NAFLD were taken as a control group in the same age and sex composition in the same community. Stool examination of all these 250 persons were done and cases of amoebiasis were diagnosed after getting at least two positive findings out of three examinations.

RESULTS

Prevalence of amoebiasis was clearly in excess in the NAFLD group in comparison to control group. This difference was tested statistically by calculating standard error of difference between two proportions and Chi-square test. The result showed a positive, strong and statistically significant association between NAFLD and intestinal amoebiasis.

CONCLUSION

There is a strong association between NAFLD and intestinal amoebiasis and further study is needed to show the biological plausibility to establish the causation.

KEYWORDS

Association, Intestinal Amoebiasis, Nonalcoholic Fatty Liver Disease (NAFLD).

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BACKGROUND

Nonalcoholic Fatty Liver Disease (NAFLD) is a condition characterised by excess accumulation of fat in the liver of people who consume little or no alcohol. It is emerging as a commonest liver disorder worldwide since its first description by Ludwig and Colleagues in 1980.¹ Other terminologies that were used for this disease entity include pseudo-alcoholic hepatitis, alcohol-like hepatitis, nonalcoholic Laennec's disease, fatty liver hepatitis, steatonecrosis, diabetes, hepatitis and nonalcoholic steatohepatitis. Nonalcoholic fatty liver disease is a spectrum of clinicopathological disease ranging from simple steatosis, Nonalcoholic Steatohepatitis (NASH), advanced fibrosis to cirrhosis of liver.² Emerging evidence is

beginning to point towards NAFLD, a hepatic manifestation of the metabolic syndrome as it shares a number of abnormalities including insulin resistance, visceral obesity, dyslipidaemia, diabetes, hypertension plus several additional factors.³ Liver enzymes ALT and AST are 2 to 4 times increased in most of the cases.⁴ Globally, the prevalence of NAFLD varies in the general adult population between 13 to 23% with higher estimates 60 to 70% in obese. Even in India, the same rising trend is being observed because of the changes in the sociodemographic and lifestyle with economic prosperity.^{5,6}

Das et al (2010)³ conducted a prospective multistaged community-based research to study the prevalence of NAFLD in population of India. The result of study found to have the high prevalence of NAFLD to the level of 8.7% in the sample rural population including 0.2% prevalence of cryptogenic cirrhosis. Steatosis was found over two third of obese population regardless of diabetes status. Steatohepatitis, a more aggressive form was seen in around 10% of subject with NAFLD. In different studies in Asian countries, prevalence of NAFLD were found varying from 2% to 27% in different group of general population.^{7,8,9} One special feature of NAFLD in developing

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countries is that non-obese population has high prevalence of NAFLD in comparison to developed countries.

Long-term prognosis of the patient depends upon the stages of NAFLD and histological changes in the liver. Non-NASH subtypes of NAFLD have good prognosis but some percentage of NASH may progress to cirrhosis, which is the third commonest cause of liver transplantation. Besides causing serious liver diseases, NAFLD is also associated with increased CVD risk. Rafiq et al (2009)¹⁰ studied a long-term mortality of a cohort of the patients of NAFLD and found that cardiovascular complications were the most common cause of death of NAFLD patients.

Normal liver fat content is 5% of the liver by weight mostly in the form of triglycerides. The liver is said to be fatty when more than 5-10% of hepatocytes show steatosis histologically. The liver derives its fat from various sources including diet, circulating Free Fatty Acid (FFA) and de novo lipid synthesis in regulated proportion that vary with the prandial status of the individual. The excess fat accumulation in the liver can be secondary to various conditions affecting the liver including alcohol, drugs, intestinal bypass procedures for weight loss, mitochondrial disease and total parenteral nutrition or maybe a part of systemic metabolic dysfunction characterised by obesity, diabetes mellitus and dyslipidaemia better known as Metabolic Syndrome (MS). High fat diet, physical inactivity, high body weight and insulin resistance are directly associated with prevalence of NAFLD.¹¹ However, fatty liver may develop in absence of any of these predisposing condition or all of these conditions. It means, there are some additional factors, which are responsible for liver injury and inflammation of hepatocytes leading to development of fatty liver condition.¹

Intestinal amoebiasis is a disease in which a protozoan parasite *Entamoeba histolytica* is harboured with or without any clinical manifestation. Symptomatic disease occurs in less than 10% of infected individuals.¹² Symptomatic group can be further divided into intestinal and extraintestinal amoebiasis. A small percentage of those having intestinal infection will develop invasive amoebiasis. Intestinal disease varies from acute fulminating dysentery to mild abdominal discomfort and diarrhoea. Extraintestinal amoebiasis includes involvement of liver, lung, brain, etc.^{12,13} In India, it is generally agreed that amoebiasis affects about 15% of Indian population ranging from 3.6% to 47.4% in different areas. Mode of transmission is mainly feco-oral route. It is closely related with poor sanitation, low socioeconomic status, hot and wet climate and personal habits.¹⁴ Though most of cases of amoebiasis remain asymptomatic, it may have subclinical or mild effect on intestinal or liver cells causing effect on fat metabolism and accumulation of fat in liver.¹⁴ It would be interesting to know the association between NAFLD and intestinal amoebiasis to get better knowledge of causation and pathogenesis of NAFLD. These two conditions are very common in North India, and if these are found associated, it may give some new angle in the understanding of these diseases in future.

Aims and Objectives

Aims and objective of this study is to know the association between an old protozoal disease, intestinal amoebiasis, which is very much prevalent in India especially in the Gangetic plain of North India and NAFLD, a new disease emerging as a modern epidemic in the whole world including India. If this association is established, then further studies can be done to see whether amoebiasis has any role in the causation or perpetuation of NAFLD. There are many unknown risk factors of NAFLD, and aim of this study is to see whether amoebiasis is one of them.

MATERIALS AND METHODS

A case-control study was done to see the association between NAFLD and intestinal amoebiasis in the city of Bhagalpur having about 5,00,000 population situated at the bank of river Ganges in the month of March-April 2017. 120 individuals, who were diagnosed cases of NAFLD were taken as a case group. Diagnosis of NAFLD was done by ultrasonography and elevation of liver enzymes Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) by more than 4 folds. These 120 cases were in the age group of 30 to 60 years including 65 males and 55 females. All patients were from the low and middle socioeconomic group of the society. 60 patients were taken from the hospital and other 60 cases were taken from the clinics and selection was done by random method.

130 persons of similar age, sex and socioeconomic group, not suffering from NAFLD (68 males and 62 females) were selected by random method from the healthy attendants of the patient to function as a control group.

In both these groups, only those persons were included who used to take no alcohol or their daily alcohol consumption less than 20 g. Three repeated stool examinations were done in all those 250 persons and individuals with at least two positive findings were taken as positive cases of amoebiasis. Diagnosis of amoebiasis was done by doing stool examination demonstrating trophozoites of *Entamoeba histolytica* in the fresh sample of stool. Serological test was not included in this experiment.

Inclusion and Exclusion Criteria

- Cases Group + Exposed- Persons having more than 10% fat deposition in liver in ultrasonography having alcohol consumption less than 20 g/day and *E. histolytica* present in stool.
- Control Group + Exposed- Persons having less than 5% fat in liver, alcohol consumption less than 20 g/day and *E. histolytica* present in stool.
- Case Group + Not Exposed- Persons having more than 10% fat deposition in liver, alcohol consumption less than 20 g/day and *E. histolytica* absent in stool sample.
- Control Group + Not Exposed- Persons having less than 5% fat in liver, alcohol consumption less than 20 g/day and *E. histolytica* absent in stool sample.

Sample was selected by random method and all ethical considerations were applied properly. Study tools applied were interview, observation, estimation of liver enzymes, ultrasonography and microscopic examinations of stool samples. Sample size of 250 people in the total population of 5,00,000 was considered big enough.

OBSERVATION AND RESULTS

Age and sex composition of both groups are almost similar. All persons were taken from the same geographical area and same socioeconomic group. All 250 persons were from the age group of 30 to 60 years. Age and sex compositions of people in two groups, group A (case group) and group B (control group) are shown in table no. 1 and 2. Therefore, age and sex factors supposed to have no effect on the result of this study.

Age Group	Male	Female	Total
30 to >40	18	15	33
40 to >50	25	22	47
50 to 60	22	18	40
Total	65	55	120

Table 1. Group A Case Group; Age and Sex Composition

Age Group	Male	Female	Total
30 to >40	22	19	41
40 to >50	30	25	55
50 to 60	16	18	34
Total	68	62	130

Table 2. Group B Control Group; Age and Sex Composition

In the case group of 120 persons, 37 persons were found positive for amoebiasis and rest 83 persons were negative for amoebiasis. Similarly, in the control group, which was made of 130 people, only 20 people were positive for amoebiasis and whereas 110 persons were negative. This result is demonstrated in table no. 3. Intestinal amoebiasis is taken as a risk factor and NAFLD is taken as a final outcome. Exposure rate of people in both group were calculated. In case group, exposure rate is 30.8%, whereas it is only 15.4% in control group. Difference in prevalence of amoebiasis and exposure rate of risk factor in the two groups was tested statistically and it was found that difference is highly significant and it cannot happen due to chance. Therefore, association between intestinal amoebiasis and NAFLD is strong, positive and statistically significant. Odd ratio was also calculated. It comes to 2.45. It means NAFLD is 2.45 times more common in patients suffering from amoebiasis than in general population.

	Case (NAFLD)- Group A	Control (No-NAFLD)- Group B
Exposed to risk factor (amoebiasis)	37 (a)	20 (b)
Not Exposed to risk factor (no amoebiasis)	83 (c)	110 (d)
Total	120 (a+c)	130 (b+d)

Table 3. A Case-Control Study of Amoebiasis and NAFLD

Test of significance- Result of the study was tested statistically by calculating standard error of difference between two proportions and Chi-square. Data was arranged in table no. 4 to show the number of people with and without amoebiasis in both case and control groups and exposure rates and non-exposure rates in two groups. Standard error of difference between two proportions was calculated. It came to 5.25. Double of S.E. = 2 x 5.25 = 10.5, which is less than the actual difference between the two proportion that is 30.8-15.4 = 15.4. Therefore, inference of this test is that intestinal amoebiasis is significantly associated with the NAFLD and it may have some role in the causation and development of NAFLD.

Group	Total Number of People	Disease Present (Amoebiasis)	Disease Absent (No-amoebiasis)	Exposure Rate	Non-Exposure Rate
A	120	37	83	30.8%	69.2%
B	130	20	110	15.4%	84.6%
Total	250	57	193	22.8%	77.2%

Table 4. Exposure Rate in Group A and B

Chi-square was also calculated from the same table. It comes to 8.46. Since number of rows and columns are two, degree of freedom will be one. For Chi-square 8.46 and degree of freedom 1, p value was derived from the Chi-square chart. It shows 'p' value is less than 0.01. Again, it shows that association between intestinal amoebiasis and NAFLD is very significant.

Appendices

Bar Chart and Pie Chart Analysis for Table 1

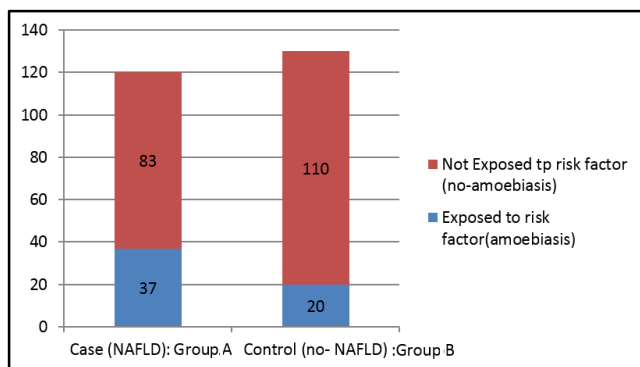


Chart 1. Exposure Rate of Group A and Group B

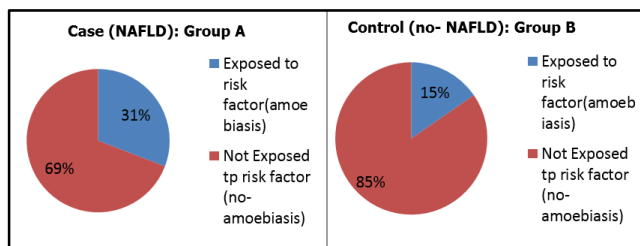


Chart 2. Exposure Rate of Group A and Group B (Pie Chart in Percentage)

DISCUSSION

The pathogenesis of NAFLD remains incompletely understood though many theories have been formulated to explain the observed sequence of events in this condition. The critical feature in NAFLD associated with metabolic syndrome is the presence of insulin resistance. It is still unknown why only steatosis develops in some of the patients at risk and furthermore why steatohepatitis and progressive disease develops in others.^{7,15} Difference in body lipid distribution or host antioxidant systems possibly in the context of genetic predisposition maybe among the explanation. The primary metabolic processes leading to fat accumulation are not well understood, but probably related to alteration of pathways of uptake, synthesis, degradation or secretion in hepatic lipid metabolism resulting from insulin resistance and liver cell injury. NAFLD is thought to be very common and the prevalence of fatty liver in general population is estimated to range from 13 to 18%. Diagnosis is done commonly by ultrasonography because it is noninvasive, simple and cheap. Ultrasonography has a specificity of 85-95% for the fat deposition and sensitivity varies with the amount of fat, 55% for 10-20% fat and 80% for >30% fat giving overall sensitivity of 65-95%. Fat deposition in the liver is classified in three grades by ultrasonography.¹⁶

Grade 1 fatty liver or mild steatosis- Increased echogenicity of liver with normal visualisation of diaphragm and intrahepatic vessels.

Grade2 fatty liver or moderate steatosis- Increase in echogenicity leading to impaired visualisation of diaphragm and intrahepatic vessels.

Grade 3 fatty liver or severe steatosis- Marked increased echogenicity and poor visualisation of posterior structures.

Alcoholic liver disease is very similar to the NAFLD, the only difference is that it is caused by excess alcohol consumption. Once the threshold level of consumption is exceeded (estimated to be 80 g/day for men and 20 g/day for women) the risk of alcoholic liver disease increases. Therefore, in this study, only those people were included who consumed no alcohol or their daily consumption was less than 20 g/day.¹

Amoebiasis is a very common infection of human GIT not only in India, but all over the world. Globally, it is estimated that 10% of the world's population carry *E. histolytica* in their intestinal tract and approximately one tenth of infected people suffer from invasive amoebiasis.^{17,18,19} In India, it is generally agreed that amoebiasis affects about 15% of Indian population, but prevalence maybe very high in areas devoid of sanitation where it may rise to 50 to 60%.^{13,18} In the present study, prevalence of amoebiasis was found to be 15.4% in the control group, which is very similar to the prevalence of general population. But, the prevalence in case group was 30.8%, which is very high in comparison to control group. The result was tested by calculating standard error (of difference) and Chi-square. The result is highly significant. Odd ratio is about 2.45. Though sample size is not very large to have very reliable odd ratio, still high odd ratio gives us strong indication of association.

Diagnosis of amoebiasis was done by demonstration of trophozoite and cyst in the fresh samples of stool of which sensitivity is 65% when one sample is taken and goes higher as the number of samples is increased. In the present study, 3 samples were taken, which can considered as very reliable.^{13,17,18,19}

Strong association between NAFLD and amoebiasis is an important finding. Though most of the cases of amoebiasis are asymptomatic, it infects intestinal and liver cells to different extent causing injury to them at different levels. What is the effect of these injuries on the lipid metabolism and whether it cause or help other risk factors in causing the fat deposition in the liver is a subject to study. However, causation cannot be established without studying biological plausibility. Further epidemiological studies (preferably cohort study) and pathological studies are needed to know the role of amoebiasis in the causation of NAFLD.

CONCLUSION

A case-control study was done in the city of Bhagalpur situated at the bank of Ganges in Bihar to know the association between intestinal amoebiasis and NAFLD and to know whether amoebiasis acts like a risk factor in development of nonalcoholic fatty liver or not. 120 NAFLD cases were taken as case group and 130 healthy persons were taken as control group and exposure of amoebiasis in these two groups were calculated, compared and tested. Result shows that these two are strongly associated,

therefore, role of amoebiasis in the development of NAFLD cannot be denied.

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