

Association of Breastfeeding with Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis in mothers: A cross-sectional survey

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ABSTRACT

Objective: To determine the association of Breastfeeding with Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis in mothers.

Methodology: A cross-sectional study on randomly selected 280 mothers presented in the outpatient department of Sheikh Zayed Hospital, Rahim Yar Khan for different medical reasons from July to Dec 2023. Data collection commenced after taking ethical approval from the Institutional Review Board of the institute and taking informed verbal consent from study subjects. These mothers were divided into 2 groups. Group 1 which never breastfed their children and group 2 which breastfed their children for more than 6 months. Each group had 140 participants. Subject having history of alcohol consumption or used medicines like glucocorticoids, amiodarone, tamoxifen, and methotrexate were excluded from the study. Biodata of all the participants along with demographic profiles were obtained, who then went through an LFT and Lipid profile blood sampling and an USG abdomen. Results were recorded on a predesigned proforma. Serum ALT, AST, Serum bilirubin, serum LDL, HDL, Triglycerides, and Serum Cholesterol were determined by enzymatic assay and high-performance liquid chromatography methods. Data was analysed using SPSS v.26. Descriptive statistics was used to summarize the data. Mean and standard deviations were reported for continuous variables. Chi square test was used to compare the qualitative variables, with p value of less than 0.05 taken as significant.

Results: Mean age \pm SD of participants was 40 ± 4 years, with group-1 having mean age of 38.1 ± 5 years and group-2 having 40.4 ± 3 years ($p=0.4$). The findings showed that frequency of NASH in patients who have not breastfed their children was higher 19 out of 140 (13.6%) whereas the mothers who breastfed their children only have 5% frequency of NASH i.e. 7 out of 140 (P value = 0.01). Out of 140 patients who did not breastfeed their children 56 (40.0%) developed NAFLD, contrary to 140 patients who breastfed their children only 20 (14.3%) (P value = 0.00) got NAFLD. Elevated ALT and AST levels are significant for NAFLD and NASH. Lipid profile is a major indicator of NAFL and NASH.

Conclusion: The frequency of NAFLD and NASH is significantly low in mothers who breastfed their children. The frequency further decreases if the period of breastfeeding was greater.

KEYWORDS: Breast feeding, Non-Alcoholic Fatty Liver Disease, Non-Alcoholic steatohepatitis.

INTRODUCTION

Breastfeeding is defined as feeding a human child from a lactating female either directly from her

breast or extracting the milk from them through a pump and bottle feeding the child later with that milk.¹ Breastfeeding is recommended exclusively; for at least six months and with other food for twelve month.² Breastfeeding is beneficial for the child, but on the other hand brings many health benefits for the mother as well.³ Breastfeeding reduces the risk of ovarian cancer by 24%, breast cancer by 20%, endometrial cancer by 11%, thyroid cancer, type 2 diabetes mellitus, cardiovascular diseases, obesity and more.^{4,5}

In Additional to its multidimensional beneficial effect on mother's health there is an underlying

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benefit of protection of the liver. Breastfeeding has been studied in relation with liver diseases in the past and there is a positive effect found.^{6,7} Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic steatohepatitis (NASH) are two most common liver conditions worldwide, and are associated with breastfeeding and lactation also.^{8,9} NAFLD is the accumulation of fat in the liver with no evidence of alcoholic or medicine interference.^{10,11} NASH is an inflammation of the liver due to this fatty tissue accumulation.¹² It can sometimes lead to cirrhosis and Chronic Liver Disease (CLD) as well. Breastfeeding and lactation play a role in preventing a mother sooner or later in her life from NAFLD and NASH. The longer the mother feeds the child, saves her from the risk of developing NAFLD and NASH.¹³

The prevalence of NAFLD in south Asian population is 26.0% and 26.6% in rural and urban communities respectively advanced liver disease is 29.3% .^{14,15} Pakistan has NAFLD prevalence of 14-47% while NASH was 12.3%.¹⁶ A major chunk of this population are females. Breastfeeding can act as a shield for women against NAFLD and NASH. Therefore, a major campaign backed by scientific evidence is required to educate women about breast feeding and its potential health benefits for mother in addition to her child. NAFLD and NASH are more common in obese, the benefit of breast feeding in reducing weight and obesity is an extra gain to mothers to avoid NASH and NAFLD through breastfeeding. Moreover, the WHO target for breastfeeding is still not achieved despite improvement and efforts in the last decade, so more efforts and data is required to convince mother about the health benefits of breastfeeding.^{16,17} This will indirectly serve the cause for promoting breast feeding and child health at the same time. The objective of this study was to determine the association of Breast feeding with Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis in mothers.

METHODOLOGY

This cross-sectional study was comprised of 280 mothers who presented in the outpatient department of Sheikh Zayed Hospital, Rahim Yar Khan for seeking healthcare for their kids or as attendants with other patients, during July to December 2023. Data collection commenced after taking ethical approval from the Institutional Review Board of the institute (ref.No 831/IRB/SZMC/SZH) letter and taking informed verbal consent from study subjects. A sample size of 278 was calculated by using Open Source Calculator for Sample Size for frequency in a population from Open Epi, version 3, in accordance with 97% confidence level. But we took 280 participants, keeping in mind if any participant will drop out due to any reason.

Enrolled mothers were divided randomly into 2 groups. Group 1 which never breastfed their children and group 2 which breastfed their children for more than 6 months. Each group had 140 participants. Study subject had a history of alcohol consumption or used medicines like glucocorticoids, amiodarone, tamoxifen, and methotrexate were excluded. All the participants, after consent, gave information about their biodata, were weighed and then went through an LFT blood sampling and USG abdomen. Results were recorded on a predesigned proforma. Liver markers including ALT (normal 7-55 IU/L, Elevated ≥ 56 IU/L), AST (normal 8-48 IU/L, elevated >49 IU/L), Serum bilirubin (normal ≤ 1 mg/dl, elevated >1.1 mg/dl), serum LDL (normal ≤ 130 mg/dl, elevated >131 mg/dl), HDL (normal ≥ 50 mg/dl, decreased <49 mg/dl), Triglycerides (normal ≤ 150 mg/dl, elevated >151 mg/dl), and Serum Cholesterol (normal ≤ 200 mg/dl, elevated >201 mg/dl) were determined. The NAFLD and NASH were determined and graded through USG using Hamaguchi score and US-FLI score.^{18,19} Hamaguchi scoring based on four components; first is bright liver, second hepatorenal echo contrast, third is vascular blurring and fourth is deep attenuation and each factor has one number. US-FLI score is based on poor vessel visibility, liver

kidney contrast, attenuation of ultrasound beam, poor gall bladder visibility, poor visibility of diaphragm and presence of fatty sparing areas. A score of more than two is significant for the diagnosis.

This data was analysed using SPSS v.26. Descriptive statistics was used to summarize the data. Mean and standard deviations were reported for continuous variables. Categorical variables were presented in frequencies and percentages. The normality of the data was checked by using Shapiro Wilk test. Chi square test was used to evaluate the difference in laboratory investigation between two groups. P-value < 0.05 were considered significant.

RESULTS

Overall total number of study subjects were 280, with mean age of 40±4 years, with group-1 having

		Breast feeding mothers		Non breast feeding mothers	
		Age		Age	
Disease		30-39	40-50	30-39	40-50
Hepatic Steatosis on USG	No	58 (86.6%)	62 (84.9%)	47 (73.4%)	37 (48.7%)
	Yes	9 (13.4%)	11 (15.1%)	17 (26.6%)	39 (51.3%)
Hepatitis on USG	No	64 (95.5%)	69 (94.5%)	59 (92.2%)	62 (81.6%)
	Yes	3 (4.5%)	4 (5.5%)	5 (7.8%)	14 (18.4%)

		Bilirubin Breast feeding mothers		Bilirubin Non breast feeding mothers	
		Normal (≤1mg/dl)	Elevated (>1.1mg/dl)	Normal (≤1mg/dl)	Elevated (>1.1mg/dl)
Hepatic Steatosis on USG	No	120 (85.7%)	0 (0%)	84 (60.0%)	0 (0.0%)
	Yes	20 (14.3%)	0 (0.0%)	56 (40.0%)	0 (0.0%)
Hepatitis on USG	No	133 (95.0%)	0 (0.0%)	121 (86.4%)	0 (0.0%)
	Yes	7 (5.0%)	0 (0.0%)	19 (13.6%)	0 (0.0%)

Table3: Comparison of AST and ALT in breast feeding and non-breast feeding with NAFLD & NASH (n= 280)

	Breast feeding mothers	Non breast feeding mothers	Breast feeding mothers	Non Breast feeding mothers	Breast feeding mothers	Non breast feeding mothers	Breast feeding mothers	Non breast feeding mothers	
	ALT Normal (7-55 IU/L)		ALT Elevated (>56 IU/L)		AST Normal (8-48 IU/L)		AST Elevated (>49 IU/L)		
Hepatic Steatosis on USG	No	119 (96.0%)	83 (83.8%)	1 (6.3%)	1 (2.4%)	120 (92.3%)	84 (77.8%)	0 (0%)	0 (0.0%)
	Yes	5 (4.0%)	16 (16.2%)	15 (93.8%)	40 (97.6%)	10 (7.7%)	24 (22.2%)	10 (100%)	32 (100.0%)
Hepatitis on USG	No	123 (99.2%)	97 (98.0%)	10 (62.5%)	24 (58.5%)	129 (99.2%)	106 (98.1%)	4 (40%)	15 (46.9%)
	Yes	1 (0.8%)	2 (2.0%)	6 (37.5%)	17 (41.5%)	1 (0.8%)	2 (1.9%)	6 (60%)	17 (53.1%)

mean age of 38.1±5 years and group-2 having 40.4±3 years (p=0.4). Age distribution of breast feeding & non-breast-feeding mothers described in table1.

Table4 (a): Comparison of Lipid profile in breast feeding and non-breast feeding with NAFLD & NASH (n= 280)

		Breast feeding mothers		Non- breast feeding mothers		Breast feeding mothers		Non- breast feeding mother	
		Triglycerides		Triglycerides		Cholesterol		Cholesterol	
		Normal (≤150 mg/dl)	Elevated (>151 mg/dl)	Normal (≤150 mg/dl)	Elevated (>151 mg/dl)	Normal (≤200 mg/dl)	Elevated (>201 mg/dl)	Normal (≤200 mg/dl)	Elevated (>201 mg/dl)
Hepatic Steatosis on USG	No	115 (93.5%)	5 (29.4%)	80 (87.0%)	4 (8.3%)	119 (90.8%)	1 (11.1%)	84 (75.7%)	0 (0.0%)
	Yes	8 (6.5%)	12 (70.6%)	12 (13.0%)	44 (91.7%)	12 (9.2%)	8 (88.9%)	27 (24.3%)	29 (100.0%)
Hepatitis on USG	No	121 (98.4%)	12 (70.6%)	88 (95.7%)	33 (68.8%)	129 (98.5%)	4 (44.4%)	105 (94.6%)	16 (55.2%)
	Yes	2 (1.6%)	5 (29.4%)	4 (4.3%)	15 (31.3%)	2 (1.5%)	5 (55.6%)	6 (5.4%)	13 (44.8%)

Table4 (b): Comparison of Lipid profile in breast feeding and non-breast feeding with NAFLD & NASH

		Breast feeding mothers		Non- breast feeding mother		Breast feeding mothers		Non- breast feeding mothers	
		LDL		LDL		HDL		HDL	
		Normal (≤130mg/dl)	Elevated (>131 mg/dl)	Normal (≤130mg/dl)	Elevated (>131 mg/dl)	Normal (≥50 mg/dl)	Decreased (<49 mg/dl)	Normal (≥50 mg/dl)	Decreased (<49 mg/dl)
Hepatic Steatosis on USG	No	117 (95.9%)	3 (16.7%)	82 (91.1%)	2 (4.0%)	120 (95.2%)	0 (0.0%)	84 (83.2%)	0 (0.0%)
	Yes	5 (4.1%)	15 (83.3%)	8 (8.9%)	48 (96.0%)	6 (4.8%)	14 (100.0%)	17 (16.8%)	39 (100.0%)
Hepatitis on USG	No	120 (98.4%)	13 (72.2%)	87 (96.7%)	34 (68.0%)	124 (98.4%)	9 (64.3%)	97 (96.0%)	24 (61.5%)
	Yes	2 (1.6%)	5 (27.8%)	3 (3.3%)	16 (32.0%)	2 (1.6%)	5 (35.7%)	4 (4.0%)	15 (38.5%)

Table 2 & 3 shows comparison of liver profile among mothers with and without breastfeeding with NAFLD and

NASH subjects.

Lipid profile of the study participants of both groups are expressed in tables 4 and b. The findings of table 5 shows that frequency of NASH in patients who have not breastfed their children is 13.6% (P value 0.01) whereas the 5% of mothers who breastfed their children only have has NASH (P value 0.01).

Group	Hepatitis on USG		Total	P value
	No2	Yes		
BF	133(95%)	7(5.0%)	140 (100%)	0.01
Non BF	121(86.4%)	19(13.6%)	140 (100%)	
Total	254(90.7%)	26(9.3%)	280 (100%)	

Pearson Chi- Square test with p value <0.05 as significant

The Findings of table 6 shows that out of 140 patients who did not breastfed their children 56 (40.0%) (P value 0.00) developed NAFLD, contrary to 140 patients who breastfed their children only 20 (14.3 %) develop NAFLD (P value 0.00).

Group	Hepatic Steatosis on USG		Total	P value
	No	Yes		
BF	120 (85.7%)	20 (14.3%)	140 (100%)	0.000
Non BF	84 (60%)	56 (40.0%)	140 (100%)	
Total	204 (72.9%)	76 (27.1%)	280 (100%)	

Pearson Chi- Square test with p value <0.05 as significant

DISCUSSION

The Data collected and compiled through the SPSS shows different attributes of disease incidence in lactating and non-lactating mothers and the association of the chemical markers like AST, ALT, LDL, HDL, etc. Our data clearly shows a strong association between lactation and decreases chances of getting NAFLD and NASH. Where the

incidence of NAFLD in normal population is around 32% worldwide.²⁰ Prevalence in Pakistan is around 47%.²¹ The statistics in our project showed prevalence of 40% in non-lactating mothers and 14.28% in lactating mothers. On the other hand, the frequency of NASH was found to be 13.57% in non-lactating mothers and 5% in lactating mothers. The factor of age seems to put an impact in the frequency of the disease too. Out of 140 non-breastfeeding mother, majority 51.31% of mothers with NAFLD lied in age ranged 40 to 50year whereas only 26.56 % NAFLD of this group were lied in age ranged between 30 to 40years. However, among breastfeeding mothers 13.4% of mothers with NAFLD fell in age ranged 30-40 years, on the other hand only 15% of NAFLD patients of this breastfeeding group were lied in aged between 40-50 years, which depicts that breastfeeding not only decreases the risk of NAFLD but also gives a long-term protection from its occurrence in later life. Eng PC et al found out that the prevalence of NAFLD is higher in males than females before menopause however the prevalence is 1.2 times higher in females after menopause.²² Furthermore the study conducted by Rizwana et al. in Islamabad and Rawalpindi, showed the prevalence of NAFLD in males 16.1% and in females to be 13.4%, with a prevalence of 19.9 in participants aged more than 40 years and 8.7% in participants aged less than 40 years.²³ It is also evident from the data the time period of which a mother breastfeeds his child also has a significance. It is seen in the data that as the number of months a mother breastfeeds increases decreases the incidence of NAFLD. The mothers who breastfed their children for 6-12 months, 22% got NAFLD, whereas the mothers who breastfed their children for 12-24 months had a lesser (10.2%) incidence of NAFLD, 24-36 months had an incidence of 4.8% and 36-48 months had an incidence of 0% according to our data. In a study done by Peter et al. the prevalence of NAFLD was 18.3%, 14.3%, 12.3%, 14.4%, and 15.8% in women with a breastfeeding period of <1, ≥1-<3, ≥3-<6, ≥6 <12, and ≥12 months, respectively.²⁴ NASH (non-

alcoholic steatohepatitis) in breast feeding mothers is relatively less prevalent than NAFLD.³ very small percentages of breastfeeding mothers disease progressed to NASH. Only 4.47 and 5.47% of NASH mothers were fall in age group 30-40 and of 40-50 years respectively. The non-lactating mothers had a comparatively higher incidence of NASH, accounting 7.8% from 30-40 years age group and 18.4% from 40-50 years age group.

Furthermore, these findings enhanced the importance of breastfeeding by throwing light on the strong association of NAFL and NASH with breastfeeding, which demonstrated a more than 50% difference of disease incidence in mothers who did not breastfeed their children. Mothers who breastfed their children showed 14.3 % incidence of NAFL and 5% incidence of NASH, on the other hand the incidence raised to 40% and 13.6% respectively in mother who did not breastfeed their children. Ajmera et al in 2019 demonstrated that the breastfeeding more than 6 months reduces the prevalence of NAFLD to more than 50% than in mothers who breastfed their children for 0-1 month.²⁵

Liver enzymes are usually deranged in liver diseases, but for NAFLD and NASH, ALT has the highest significance and has a prevalence of 4% to 21.7% and, on its bases, an early and prompt diagnosis of NAFLD can be made.^{26,27} In our study, we also included three liver enzymes as the marker of NAFLD i.e. ALT, AST and serum bilirubin. According to our results, among the non-lactating mothers, majority 97.3% had NAFLD with elevated ALT levels and only 16.2% had NAFLD with normal ALT levels. Similarly, majority 41.5% had NASH with elevated ALT levels and only 2% had NASH with normal ALT levels. Same as non breastfeeding group, among the breastfeeding mothers, majority 93.8% of NAFLD mothers had elevated ALT levels and only 4% had normal ALT levels. Concerning NASH in breast feeding mother 37.5% with elevated ALT levels whereas 0.8% had NASH with normal ALT levels shows significance of liver enzymes in liver disease. Our data

demonstrates a correlation of elevated levels of AST with both NAFLD and NASH. We found raised of AST in majority NAFLD and NASH patients of both non breast feeding as well as breast feeding mothers, only few patients had normal AST levels. Current findings are quite comparable with a prevalence of 32% in another study.²⁷ This aforementioned study had also reported elevated levels of AST and ALT in NAFLD and NASH.²⁷

A deranged lipid profile is strongly associated with NAFLD and NASH, and is, in fact, one of the key chemical indicators of the disease. High LDL, triglycerides, and low HDL levels all correlate with the incidence and progression of NAFLD.²⁷ In current study 96% and 91.7% of NAFLD patients in nonlactating mothers had elevated LDL and triglyceride levels respectively. On contrary to this HDL levels in majority of the NAFLD patients were lower and only few numbers of patients had normal HDL levels. Current study also reveals elevated LDL, TG and cholesterol levels in 32, 31.3% and 44.8% of NASH patients of nonlactating group of mothers respectively whereas, on analyzing the lipid profile of breast-feeding mothers, we also found elevated liver markers including LDL, TG and cholesterol levels in majority of the NAFLD as well as NASH patients of this group. Concerning HDL, lower levels in majority of the NAFLD patients, however HDL levels were normal in NASH patients of both lactating and non-lactating mothers, these finding are justified by with previous similar studies, those also reported high LDL, TGs, cholesterol and low HDL are strongly associated with NAFLD and NASH.^{28,29,30}

Limitation: Small sample size is the limitation of the study so results were not generalized to whole population.

CONCLUSION

There is a strong association between breastfeeding and NAFLD and NASH as the frequency of NAFLD and NASH is significantly low in mothers

who breastfed their children. Elevated ALT and AST levels are significant for NAFLD and NASH.

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Ghulam Mustafa: Study design, Data Acquisition, Data Analysis and Interpretation revised, drafting manuscript and approved the manuscript.

Manzur Ahmad Manzur: Data Acquisition, manuscript writing, revised and approved the manuscript.

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