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ORIGINAL ARTICLE

A Comparison of Treatments Offered to Patients with Nonalcoholic Steatohepatitis

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ABSTRACT

Objective: To compare various treatment options provided to patients with Nonalcoholic Steatohepatitis (NASH) and assess improvement in liver status via reduction in serum Alanine Aminotransferase (ALT) levels.

Study Design: Retrospective cohort study.

Place and Duration of Study: The Aga Khan University Hospital, Karachi, from April 2000 to April 2007.

Methodology: All available records of patients aged between 20-70 years, fatty liver on ultrasound, elevated serum ALT and having at least one follow-up, after a baseline visit were included. The patients had variable number of follow-ups and a maximum of 3 follow-ups were considered. Information was collected on demographic and clinical characteristics of the subjects. The treatment options were categorized as weight reduction alone, with statins, and with other medications. Serum ALT level was the main outcome measured in IU/l. Repeated-measures ANOVA, using a mixed model approach was performed with treatment options as between subject factor, and follow-up as within subject factor and p-value < 0.05 was considered significant.

Results: Sixty-nine records of subjects, consisting of 50 males and 19 females were selected. The mean (± SD) age was 40±12 years. Thirty-one subjects (45%) were advised weight reduction only, and experienced a 72% reduction in serum ALT levels, over the mean follow-up time of 9±3 months. Twelve subjects (17%) received statins along with weight reducing advice, and experienced a 56% reduction in mean ALT over the mean follow-up of 11±7 months. Twenty-six subjects (38%) received other medications along with advice for weight reduction and experienced a 73% reduction in serum ALT levels over the mean time of 10±4 months.

The mean ALT declined at follow-up times, irrespective of the prescribed treatment, and that the decline with time was different for males and females.

Conclusion: Serum ALT levels among patients with NASH decreased with time, regardless of the provided treatment, and the decrease was different for males and females.

Key words: *Nonalcoholic steatohepatitis (NASH). Fatty liver. Alanine aminotransferase.*

INTRODUCTION

Non-alcoholic Fatty Liver Disease (NAFLD) has become an important disease entity owing to its common occurrence in the general population and its potential to progress to cirrhosis and hepatic failure. Nonalcoholic steatohepatitis (NASH) is a form of NAFLD, which refers to a wide spectrum of liver damage, ranging from simple steatosis to advanced steatohepatitis and cirrhosis.

Steatosis (fatty liver) is an accumulation of fat in the liver, mostly triglycerides, exceeding 5-10% of the weight of the liver.[1] It is caused by a failure of normal hepatic fat metabolism due to a defect either within the hepatocyte or in the delivery of lipids from the liver cells.[2] Symptoms may include fatigue or malaise, and a sensation of fullness or discomfort on the right side of the upper abdomen.[3] Hepatomegaly is a common feature. Serum alanine aminotransferase may be normal or high. Liver biopsy is the gold standard technique to diagnose steatosis and steatohepatitis, but it is an invasive procedure and associated with many complications, which may become fatal. Imaging techniques, like ultrasonography, though not the Gold standard, are effective to diagnose fatty liver.[3,4] Research shows that ultrasonography detects fatty liver changes in 12.9%-16.4% of individuals.[5]

NAFLD affects all age groups; the greater predominance is seen among male adults.[6-8] The prevalence of NAFLD averages about 10-30%, and that of NASH is 2-3% of the general population in many countries.[1,5] The prevalence may increase to approximately 50-70% in the presence of risk factors such as obesity, Diabetes mellitus, and dyslipidemia.[3,4] An increase in body weight is well correlated with the degree of fatty liver.[4] Few studies are in the context with the Asian Body Mass Index (BMI), which defines new cut off points for Asians.[9]

The pathogenetic mechanisms behind NASH progression are not well-understood. However, it has

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been suggested that hepatic fatty infiltration is a consequence of increased splanchnic lipolysis and hepatic insulin resistance.[10] Researchers suggest that alteration of local and systemic factors, particularly insulin resistance, that control the balance between the synthesis of hepatic lipids and their oxidation lead to hepatic triglyceride accumulation.[5] This results in hepatocellular inflammation and fibrosis. Other factors like oxidative stress, adipocytokines, may provide secondary insults, further complicating to steato-hepatitis.[11]

Many factors have been identified to be associated with NAFLD. It is commonly associated with metabolic syndrome.[4,12] It is reported that 60-95% of the NAFLD patients are obese.[4] Other causes may include nutritional (starvation, total parenteral nutrition), medicines (like amiodarone, corticosteroids, methotrexate), and diseases (like HIV infection, inflammatory bowel disease etc.).[3,5] It is conceivable from regional data that NAFLD and NASH may also be very common in Pakistan though no community-based studies have been carried out.

Currently, the treatment options for NASH are very limited. The treatment modalities prescribed are focused on modifying the associated conditions such as obesity, dyslipidemia, and Diabetes mellitus.[1,4,10] Weight loss through diet restriction and exercise has proved to be effective in various studies.[3,5,10] The use of statins, insulin sensitizing agents, anti-oxidants (like vitamin E), and hepato-protective agents (such as pentoxifylline and ursodeoxycholic acid), as a treatment modality for NAFLD, are still being evaluated.[1,10] There have been a couple of reports on the use of Danning Pian (a Chinese herbal medicine), and choline containing foods in the treatment of NAFLD.[13,14]

This study attempts to compare various treatment options being provided to patients with NASH, attending the Aga Khan University Hospital (AKUH), Karachi, and assess liver improvement through reduction in their mean serum Alanine Aminotransferase (ALT) levels overtime.

METHODOLOGY

A retrospective cohort design was used to compare various therapeutic options provided to patients with NASH who attended the AKUH during the 7-year period (April 2000 – April 2007), using the recorded data collected from the Department of Health Information Management Services, AKUH. The treatment options were categorized as weight reduction only through the advice of dietary restriction and engagement in regular physical exercise, weight reduction and statins (commonly prescribed were Simvastatin and Atorvastatin), and weight reduction and other medications, which were ursodeoxycholic acid (30.6%), pioglitazone (22.4%), metformin (36.7%), and vitamin E (10.2%).

The diagnosis of NASH had been considered by the attending gastroenterologist, based on unexplained elevations in serum ALT, fatty liver on ultrasound and upon excluding liver metabolic disorders (hemochromatosis, Wilson's disease), auto-immune hepatitis and viral infection.[15] Serum ALT, one of the main liver function enzymes, is of main interest towards assessing improvement in liver status. This is measured quantitatively in IU/l, recorded on at least one subsequent follow-up after the initial visit. A maximum of 3 follow-ups were considered.

The inclusion criteria was to include all medical records of the patients with NASH, with the age range of 20-70 years; fatty liver on ultrasound; and raised serum ALT levels (for males greater than 55 IU/l; for females greater than 33 IU/l). The exclusion criteria were pregnancy, recent gastrointestinal surgery, history of alcohol consumption, transfusion, drug abuse, and acute/chronic infectious hepatitis, liver disorders, which would account for steatosis other than NASH (Wilson's disease, hemochromatosis) and drugs which may also result in liver steatosis (corticosteroids, methotrexate, aspirin, etc.). Moreover, those individuals with no follow-up after a baseline visit were also excluded from the study.

Height and weight was measured in SI units. The BMI was assessed, according to the Asian standards, and defined as < 18.5 kg/m^2 as underweight, 18.5-23 kg/m^2 as acceptable and 23.1-25 kg/m^2 as overweight.[9] Patients were also assessed for other comorbids such as hypertension (systolic blood pressure exceeding 140 mmHg, and/or diastolic blood pressure exceeding 90 mmHg) and Diabetes mellitus (fasting serum glucose levels exceeding 110 mg%).

Serum ALT levels were the main outcome of interest measured in IU/l. This was assessed on a quantitative scale, among subjects with at least one follow-up after the baseline visit. Demographic variables included age, gender and marital status. Baseline characteristics included patient history and family history of comorbid conditions, such as Diabetes mellitus, hypertension, dyslipidemia and hepatitis. Physical and laboratory investigations included the physical components like blood pressures, height and weight. The laboratory investigations, in addition to liver function tests (serum aspartate transaminase, ALT, and gamma glutamyl transferase), also included total lipid profile (serum levels of total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and serum glucose levels (random and fasting serum glucose).

In order to detect a minimum difference of 25 IU/l in the ALT levels at last follow-up among the 3 treatment groups at 80% power and 5% significance level, a

minimum sample of 21 subjects per group was required. The study was approved by the Ethics Review Committee of the Aga Khan University.

Data analysis was performed, using SPSS software for Windows version 15 (SPSS Inc., Chicago, IL). Means and standard deviations were calculated for quantitative variables, and proportions for the categorical variables. Baseline variables were compared among the prescribed treatment options using one-way analysis of variance. Non-parametric procedures were used for non-normal data. Repeated-measures analysis of variance, using a linear mixed model approach, was performed with the treatment options as between subject factor, and follow-up times as within subject factor.[15] Considering that the study subjects had different number of follow-ups, it was particularly relevant for this study to employ the mixed model approach. The linear mixed model analyzes data in a relational (longitudinal) format, permits flexibility in modelling variance-covariance structures, and allows for the analysis for variable number of follow-ups for each patient. Serum ALT was the outcome assessed through this approach. P-values at or < 0.05 were considered significant.

RESULTS

Eighty-two records of patients were identified who attended the hospital facility during the 7-year period (April 2000 – April 2007), and were assessed for NASH. Out of 82, 13 records were excluded for not meeting the selection criteria. To avoid any potential selection bias, the baseline demographic and clinical variables of the excluded subjects were compared with those included in the study (data not shown). The analysis demonstrated that the major demographic and clinical characteristics of the excluded subjects were not statistically different from those included in the study ($p > 0.05$). The remaining records (69) were analyzed for assessing the outcome overtime.

Table I summarizes the frequency distribution of the demographic and clinical variables. Out of 69, 50 (72%) were males and 19 (28%) were females. Out of the total 65 (94%) were overweight, 9 (13%) were hypertensive, 44 (64%) were diabetic, and 48 (70%) had abnormal serum lipid levels. Thirty-one out of 69 (45%) received the treatment of weight reduction only, 12 (17%) received statins along with advice for weight reduction, and 26 (38%) received other medications along with weight reducing advice.

All subjects came with the primary concern of having raised serum ALT on routine check-ups. In addition, 55% of the total also reported to have abdominal pain and about 35% of the cases complained about fatigue and weakness. Percentages of positive family history for obesity, dyslipidemia, hepatitis, hypertension and diabetes mellitus among cases were 37, 38, 40, 45 and 45, respectively.

Table I: Frequency distribution of demographic and clinical characteristics.

Variables	Number (%)
Sex	
Male	50 (72)
Female	19 (28)
Comorbids*	
Hypertension	9 (13)
Overweight	65 (94)
Diabetes mellitus	44 (64)
Dyslipidemia	48 (70)
Treatment option	
Weight reduction only	31 (45)
Weight reduction and statins	12 (17)
Weight reduction and other medications	26 (38)
Presenting complaints*	
Raised serum ALT	69 (100)
Abdominal pain	38 (55)
Malaise and fatigue	24 (35)
Family history*	
Obesity	26 (37)
Dyslipidemia	27 (38)
Hypertension	31 (45)
Diabetes mellitus	31 (45)
Hepatitis	28 (40)

** Multiple responses*

Tables II summarizes the clinical characteristics of patients at baseline and at various follow-ups, for each of the 3 prescribed treatments; weight reduction only, weight reduction and statins, and weight reduction and other medications, respectively. The mean age of the group advised with weight reduction only was less than that for the groups receiving statins and other medications, in addition to weight reducing advice ($p=0.033$; Table II). Mean systolic blood pressures at baseline in the group receiving drugs along with weight reduction were significantly greater than the group receiving weight reducing advice only ($p=0.003$; Table II). Similarly, mean diastolic pressures at baseline in the group receiving other medications along with weight reduction was also greater than the other groups (p-value=0.047; Table II). This shows that the groups receiving medications were relatively older and had comorbids like hypertension. All the other clinical characteristics were comparable among the 3 treatment modalities at baseline.

A comparison of the subgroups receiving medications other than statins revealed that mean baseline ALT levels was highest in the groups receiving ursodeoxycholic acid compared to subgroups on pioglitazone, metformin and vitamin E (Table IIA). However, the values reached the normal values in all the subgroups within the 3rd follow-up. No other significant change was observed in other parameters of these subgroups.

A declining trend overtime is observed for serum ALT levels in all treatment groups (Figure 1). The group being advised with weight reduction only showed a 72% reduction in their serum ALT levels over the follow-up

Table II: Clinical characteristics of patients receiving various treatments at baseline and various follow-ups (mean±SD).

	Weight reduction only				Weight reduction and statins				Weight reduction and other medications			
Variable*	Baseline	Follow-up 1	Follow-up 2	Follow-up 3	Baseline	Follow-up 1	Follow-up 2	Follow-up 3	Baseline	Follow-up 1	Follow-up 2	Follow-up 3
Age (years)	36±9	-	-	-	46±15	-	-	-	43±12	-	-	-
Systolic blood pressure (mmHg)	117±13	118±14	115±8	94±5	131±13	126±14	119±18	135±7	128±13	132±15	125±14	118±16
Diastolic blood pressure (mmHg)	77±9	76±8	74±5	63±6	77±9	71±8	79±9	75±7	83±8	84±7	81±10	78±12
Weight (kg)	79±14	78±22	78±24	78±23	83±16	70±1	76±1	78±2	82±14	82±16	82±17	82±18
Body mass index (kg/m^2)	29±5	28±7	28±8	28±7	29±4	29±2	30±5	34±5	30±5	30±5	30±5	31±5
ALT (IU/l)	95±40	57±31	46±24	26±16	101±55	76±33	60±26	44±20	90±38	56±27	35±15	30±12
AST (IU/l)	59±26	53±9	53±37	VSN	91±34	VSN	32±4	VSN	49±13	40±7	50±13	38±8
Total cholesterol (mg%)	181±68	199±65	183±53	VSN	203±70	233±50	190±79	126±117	197±23	178±51	154±32	VSN
Triglycerides (mg%)	163±95	169±51	147±52	VSN	142±102	334±299	294±257	171±198	206±104	179±81	177±55	VSN
LDL-cholesterol (mg%)	104±47	97±20	101±44	VSN	123±53	132±49	80±41	VSN	109±37	120±31	VSN	VSN
HDL-cholesterol (mg%)	41±14	44±12	VSN	VSN	38±5	43±19	36±9	VSN	41±8	43±13	VSN	VSN
Random serum glucose (mg%)	VSN	VSN	VSN	VSN	VSN	VSN	VSN	VSN	VSN	293±179	260±109	VSN
Fasting serum glucose (mg%)	VSN	VSN	VSN	VSN	VSN	139±56	116±24	VSN	VSN	161±45	VSN	68±81

** Mean age, systolic blood pressure and diastolic blood pressure were significantly different among the 3 groups (p value < 0.05)*

*** VSN = very small number of observations*

Table II A: Clinical characteristics of patients receiving other medications at baseline and at various follow-ups (means±SD).

	Ursodeoxycholic acid				Pioglitazone			Metformin				Vitamin E		
Variable	Baseline	Follow-up 1	Follow-up 2	Follow-up 3	Baseline	Follow-up 1`	Follow-up 2	Baseline	Follow-up 1	Follow-up 2	Follow-up 3	Baseline	Follow-up 1	Follow-up 2
Age (years)	43±13	-	-	-	47±17	-	-	43±12	-	-	-	38±12	-	-
Systolic blood pressure (mmHg)	126±13	133±15	126±12	126 ± 13	130±14	125±7	135±7	130±16	124±20	118±18	126±15	130±14	128±24	135±21
Diastolic blood pressure (mmHg)	85±8	86±8	84±8	84±9	80±11	75±7	80±14	84±9	81±8	75±12	69±10	86±10	85±13	88±11
Weight (kg)	83±11	83±11	83±14	90±16	88±23	83±20	84±20	79±16	78±16	78±18	67±10	85±5	84±5	85±7
Body mass index (kg/m^2)	30±5	30±4	30±5	31±5	30±6	29±7	29±7	31±6	30±6	31±6	28±5	33±2	31±2	33±3
ALT (IU/l)	110±40	70±31	42±20	32±12	66±8	41±19	31±25	66±22	43±18	30±19	VSN	89±35	62±24	28±6

Table III: Mixed model analysis: fixed effects for ALT, adjusted for age and sex.

Dependent variable	Covariance structure	P-value
ALT	Unstructured	
Age		0.195
Sex		0.011
Treatment		0.352
Follow-up time		< 0.001
Sex * treatment		0.974
Sex * follow-up time		0.045
Treatment * follow-up time		0.182

mean time of 9±3 months. The group being prescribed with statins along with weight reducing advice showed a 56% reduction over a mean time of 11±7 months, while the group receiving advice for weight reduction and other medications experienced a 73% reduction in serum ALT levels over a mean follow-up time of 10±4 months.

Mixed model analysis was performed with ALT as the outcome (Table III). The model was adjusted for age and gender, as the treatment groups differed with respect to age at baseline, and the cut offs for raised ALT differed for males and females. The mixed effect model shows that the effect of follow-up times on mean ALT was different for males and females (p=0.045). Males encountered a 60% reduction in serum mean ALT levels, while in females it got reduced by 64%. There was no significant difference in mean ALT levels in the 3 treatment groups (p=0.352) and the mean ALT levels decreased significantly overtime (p<0.001). No significant interaction was observed between follow-up times and the prescribed treatment (p=0.182) implying that the reduction in mean serum ALT levels overtime is not different for the different treatment options.

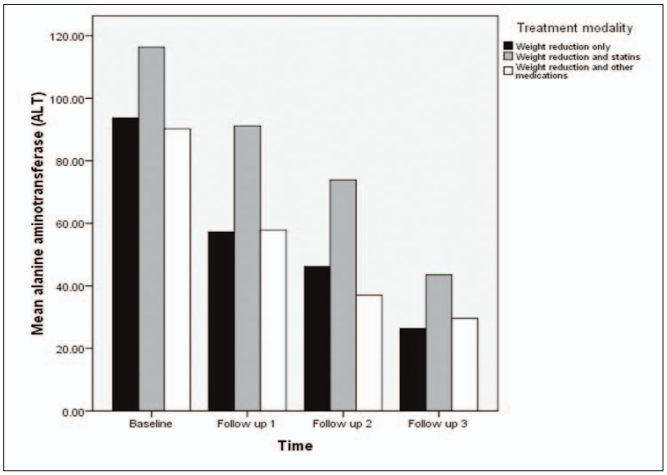

Figure 1: Mean alanine aminotransferase (ALT) at baseline and different follow-ups with respect to treatment options.

DISCUSSION

The primary objective of this study was to compare various therapeutic options being provided to Pakistani patients with nonalcoholic steatohepatitis and to assess liver function improvement through reduction in serum ALT levels. With the exception of a few small studies carried out in India and Iran,[16-18] there is hardly any research data on this subject from this part of the world. No research study of this kind has been conducted in Pakistan. This study was carried out to derive conclusions on assessing improvement in fatty liver status with respect to treatment in Pakistani patients.

Majority of the patients in this study (n=31, 45%) received the option of weight reduction only, which remains the cornerstone treatment for nonalcoholic steatohepatitis.[3,19] In the presence of other co-morbid conditions, pharmacological agents in addition to weight reducing advice would be considered beneficial for symptomatic patients.

A significant decreasing trend in serum ALT levels with follow-up time signifies improvement in NASH, irrespective of the prescribed therapeutic modality. Studies conducted elsewhere have also shown a similar trend. For example, two small studies from India and Iran showed a significant decline in serum ALT levels with respect to time ($p < 0.05$) in different treatment groups.[17,18] A similar decline in liver enzymes has also been reported in other studies carried out in some of the developed countries.[1,10,19] According to Marchesini *et al.* weight reduction remains the treatment of choice for patients with obesity.[10] Since most of NASH patients in this study were overweight, it was expected that a reduction in BMI would occur in all 3 groups and would correspond to the decline in serum ALT levels. However, no decline in BMI was observed over a mean period of 10±5 months in all the three groups. Poor compliance on the part of the patients to prescriptive advice could have been the reason for little change in mean BMI. However, this was not unique to our patient population. Similar findings have also been reported in other studies.[1,4,18,20]

NASH patients usually present with vague symptoms.[3] In this study, the presentation of the disease was asymptomatic in majority of the cases, while some reported with mild abdominal pain and fatigue. Male preponderance observed in this study has also been reported by others.[5,17] This could be due to genetic differences among various populations or females from the subcontinent might have been more hesitant in seeking treatment for a relatively asymptomatic disease, like NASH. Bahrami *et al.* suggested that exclusion of subjects with hepatitis might have been the reason for the gender differences.[17] In the present study too the diagnosis of NASH was made on patients after ruling out hepatitis.

Dyslipidemia (70%) and Diabetes mellitus (64%) were common in the study subjects. This is also consistent with other similar studies.[17,19,20] Among diabetics, data suggested that about 60% were using diabetic medications, metformin and pioglitazone, separately or combined. However, this may not have affected the progression of NASH as those latter medications are recommended for treatment of this disease.

The data revealed a number of risk factors (obesity, hypertension, diabetes, dyslipidemia), which contributes to NASH.[20-22] A recent study conducted in a tertiary care hospital in Islamabad showed an increased prevalence of metabolic syndrome,[23] a primary risk factor for NASH. Since the individual components of metabolic syndrome are reaching epidemic proportions in Pakistan, hence the prevalence of NASH would also be expected to be high in our population.

No significant decline in BMI overtime in this study merits some discussion. It is suggestive that greater follow-up time would, perhaps, be necessary to examine any significant change in BMI due to treatment. Bugianesi *et al.* in their trial followed their subjects for one year, with a 3 month follow-up time, and demonstrated a declining trend in BMI.[19] Georgescu *et al.* followed their subjects for 37-38 weeks (8-9 months), and observed a slight declining trend.[1] By extending the follow-up time, and ensuring better compliance by patients would, perhaps, be necessary to evaluate any beneficial effects of weight reduction in NASH.

There were a few limitations of this study. Being a retrospective cohort study, it was not unusual to find some laboratory values like serum ALT, AST, serum cholesterol, serum triglycerides, serum fasting and random glucose levels to be missing at consecutive follow-up times. In order to address the issue of missing values at follow-up times, the mixed model analysis was performed for ALT, as the data measurements for this variable were sufficient to conduct this advanced analysis. For the other laboratory markers of liver function, the amount of missing data was comparatively larger and so mixed model analyses could not be performed.

Recording of data on the treatment options for NASH offered another limitation to the study. Generally, prescriptions on weight reduction tend to be more verbose compared to that of drugs, which may result in misclassification of the subjects. However, for the patients assessed for NASH in this study, such advices and prescriptions were mostly available on record, thus minimizing misclassification.

As mentioned earlier, no proven therapy exists for NASH, and so there is much debate on the options of treatment as well as their duration. Assessment of compliance of subjects to the provided treatment is also

questionable, as there may be a possibility for subjects' crossing over on the provided options for treatment.

Serum ALT was used as a laboratory surrogate marker to evaluate improvement in NASH. Though changes in serum ALT can be useful as a surrogate in screening therapies for NASH with baseline ALT levels and histology,[24] however, using this as the only laboratory surrogate may be considered as another limitation. Previous research reports suggest that other liver function enzymes, especially AST, also assist in identifying steatosis and steatohepatitis.[20] We attempted to incorporate other liver function enzymes, but the missing data did not permit us to do that. Since serum levels for both AST and ALT fluctuate, therefore, variability is anticipated. Other non-invasive markers, like serum hyaluronic acid, ketoisocaproate breath test, are still under research, and may provide better directions towards assessing liver improvement in NASH, non-invasively.[25,26] According to Portincasa *et al.* liver breath testing using ketoisocaproate and methacetin isotopes can be clinically useful to characterize NASH.[25]

Mean ages of patients among the three treatment groups were not comparable. However, the mixed model analysis allowed us to adjust for age in the final effect model. Variability in the follow-up time intervals for various patients and small sample size in the group receiving weight reduction and statins were other limitations in the study. The small sample size resulted in decreased power and, therefore, the mixed model analysis may not have been able to detect significant differences with respect to the treatment groups. A prospective study using a large number of well-defined NASH patients and a follow-up of greater than 12 months would, perhaps, be necessary to ascertain whether pharmacological treatment in addition to weight reduction has any advantage over the standard weight reduction treatment in Pakistani population.

CONCLUSION

Serum ALT levels among patients with NASH decreased significantly with time, irrespective of the provided treatment option, and the decline differed between males and females. More epidemiological research in this field would be essentially required to understand the nature of disease in this part of the world and to offer better treatment options.

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