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Original Article

Effect of Pioglitazone on Non-Alcoholic Fatty Liver Disease in Type 2 Diabetic Patients Using NAFLD Fibrosis Score

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ABSTRACT
Introduction- NAFLD ranges from Simple fatty liver to NASH, Cirrhosis and hepatocellular carcinoma. Identification of patient in early stages provides possibility of disease reversal. Lifestyle modifications+ known to achieve reversal are difficult and have poor compliance. Studies have shown some effects of drugs like pioglitazone, Vitamin E, Omega-3, Metformin and urodeoxycholic acid. There are limited studies of effect of pioglitazone on NAFLD in Patients with Type 2 DM and none in Indian patients as per our literature search. **Methodology-** Total 150 patients with as per inclusion and exclusion criteria were included in the study & patients were started on pioglitazone therapy. Before starting of therapy, baseline NAFLD score was calculated for each patients. After 6 months therapy with pioglitazone again NAFLD fibrosis score was calculated for every patients. **Result-** Total 150 patients of NAFLD were given pioglitazone NFS was calculated before & after 6 month. Observations show Male: Female ratio of study subjects 3.5:1. Mean age of study subjects was 53.08 ± 6.94, NAFLD Fibrosis Score significantly decreased after treatment with pioglitazone ($p<0.01$), There was significant negative correlation between various anthropometric and biochemical parameters and NAFLD fibrosis score. ($p<0.01$) **Conclusion-**The administration of pioglitazone led to metabolic improvement in type II diabetic subjects with NAFLD. NAFLD fibrosis score was very useful non-invasive tool to assess fibrosis in NAFLD in type II diabetic subjects. Larger controlled trials of longer duration are warranted to assess the long-term clinical benefit of pioglitazone.
Key words: Diabetes, Fatty Liver, Fibrosis Score.

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INTRODUCTION-

The prevalence of non-alcoholic fatty liver disease (NAFLD) varies widely depending on the population studied and the methodology applied. Studies have shown that NAFLD may be present in up to 70% of patients with diabetes(1)(2)whilst the prevalence of biopsy proven non-alcoholic steatohepatitis (NASH) in asymptomatic type 2 diabetics with normal liver function tests (LFTs) was 20%(3). Estimates from number of studies have suggested that there is a significant burden of advanced fibrosis in asymptomatic individuals with type 2 diabetes ranging from 5% to 7%(4)(5). There is therefore no doubt that these two common conditions co-exist and that there is significant amount of unrecognized advanced NAFLD within asymptomatic diabetic patients. Obesity and physical inactivity are interlinked risk factors for the development of diabetes and both are clearly implicated in an individual's risk of developing NAFLD. In a large cross sectional study an individual's sitting time was associated with NAFLD diagnosed using US and interestingly this association held true in those with a normal BMI(6). Obesity is well known to correlate with both NAFLD prevalence and severity. In a study of patients who had liver biopsies whilst undergoing elective abdominal surgery the BMI was strongly correlated with NASH(7) and in a separate study intraabdominal fat was associated with NASH(8)(9)

NAFLD (diagnosed on ultrasound and excluding other causes of liver disease) increases the risk of cardiovascular events by 1.87-fold of an individual with type 2 diabetes after adjusting for confounders(10). Although a separate study did not identify increased mortality, in this retrospective analysis the cohort investigated was composed of those who underwent CT scanning for a specific clinical indication and this may have had an additive effect on mortality risk, potentially masking any impact of NAFLD.(11) It is important to recognise that neither of these studies used liver biopsies and as a consequence was notable to differentiate between NAFLD and NASH which may be relevant to cardiovascular disease risk(12). As well as cardiovascular risk(10), co-existent NAFLD increases the risk of microvascular complications of diabetes including chronic kidney disease and retinopathy(13). Furthermore, hepatic fat content has been shown to be associated with increased insulin requirements(14)which have the potential to fuel weight gain. The available data linking NAFLD to diabetes complications are limited in that they are mostly taken either retrospectively or from observational cohort studies rather than from longitudinal data.(9)

There is emerging evidence demonstrating an additive detrimental liver outcome for people with co-existent diabetes and NAFLD. A diagnosis of diabetes makes an individual more likely to have more severe NAFLD with the associated complications of cirrhosis and mortality. In one large cohort study, the standardized mortality ratio from cirrhosis was increased in diabetics (2.52)(15). Furthermore, in a series of 432 patients with biopsy proven NAFLD the presence of co-existent type 2 diabetes was found to be an independent risk factor for fibrosis(16). Other smaller studies that included liver biopsies have identified an additive effect of NAFLD and diabetes on cirrhosis, liver and all-cause mortality(17). In another study, those patients with periportal–portal fibrosis were more likely to have diabetes(18).In studies using serial biopsies those with progressive fibrosis were more likely to be diabetic at baseline and were also more likely to develop diabetes if not already diagnosed(19). Finally,in a meta-analysis, co-existent diabetes was associated with a poorer prognosis in individuals with hepatocellular carcinoma(20). Overall therefore, the evidence seems clear that co-existent NAFLD and diabetes are associated with a more severe adverse outcome than either of the conditions in isolation.(9)

Thiazolidinediones (TZDs) are peroxisomal proliferator activated receptor-c (PPAR-c) agonists, a class of nuclear transcription factors that are very abundant in adipose tissue. In patients with NASH, they reduce subclinical inflammation, improve adipose tissue and hepatic insulin sensitivity, and restore liver histology(21)(22). Several relatively small RCTs have demonstrated the efficacy of TZDs in patients with steato hepatitis(23)(24)(25)(26). In the only study in patients with prediabetes or T2DM and NASH(23), pioglitazone (45 mg/day) significantly diminished insulin resistance at the level of the liver, adipose tissue and muscle and improved liver steatosis, necroinflammation and hepatocellular ballooning when compared with placebo. The NASH improved in 73 % ofpatients treated with pioglitazone compared with 24 % inthe placebo group (p\0.001). Two RTCs in patients with NASH and without T2DM later confirmed these findings(24)(25). In the largest of these studies, 247 subjects were randomized to vitamin E, pioglitazone or placebo(24).They found histological improvement in liver steatosis and inflammation but not fibrosis after pioglitazone treatment. Unfortunately, the studies have been of relatively short duration (6–24 months) and confirmation about their long term benefit is needed. Moreover, the long-term safety of TZDs (heart failure, bone loss and bladder cancer) has been under much debate. Regarding bladder cancer, the FDA currently recommends avoidance of pioglitazone if active bladder cancer is present, and caution if there is prior history of the disease. On the other hand, pioglitazone has been shown to reduce CVD in patients with T2DM(27). The Asia-Pacific and the Italian guidelines acknowledge the potential benefits of pioglitazone, however, suggest that more evidence should be available before a firm recommendation can be made.(28)(29)(30)

The gold standard technique not only to diagnose, but also to determine the severity of NAFLD, is liver biopsy and histopathological examination of the specimen. Nonetheless, as it is an invasive technique, it has been a debate topic and the most recent guidelines recommend the use of liver biopsy in the diagnosis and staging of NASH. The ideal target would be the discovery of non-invasive scores to better determine the grade of steatosis and fibrosis in NAFLD, which would be not only inexpensive, but also easy to perform. For that purpose, many non-invasive scores have been proposed for NAFLD detection and staging.(31)

In our study we have used NFALD fibrosis sore to assess severity of NAFLD before and after treatment with pioglitazone. This study was aimed to evaluateeffects of pioglitazone on non-alcoholic fatty liver disease in type 2 diabetic patients using NAFLD fibrosis score.

METHODOLOGY -This Prospective interventional study was conducted over 3 years at a D.Y. Patil Hospital & Research Institute, Kolhapur and Maharashtra, India. Approval of the Institutional Ethics Committee was taken. Type 2 diabetic patients diagnosed with nonalcoholic fatty liver disease on ultrasonography were selected with exclusion of critically ill patients, patients with multi-system disorders or diagnosed viral hepatitis, those on hepatotoxic drugs and history of hepatobiliary surgery.

Informed consent was taken from all patients for use of their data. Total of 150 patients were included in the study.

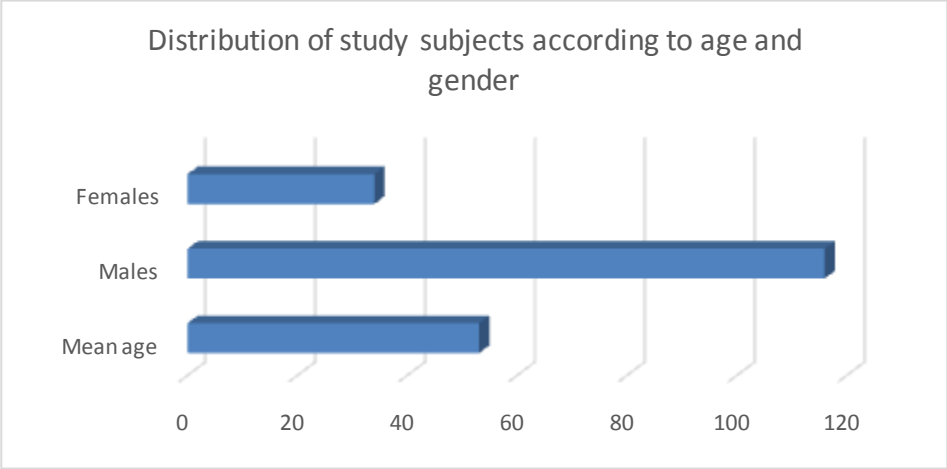
History (including family corroboration of alcohol intake where required), detailed examination, anthropometric measurements, Complete Blood Count, Liver Function Tests were carried out in the study participants. NAFLD Fibrosis Score calculated all patient were started on pioglitazone therapy. Before starting of therapy baseline NAFLD score was calculated for each patients. After 6 month therapy with pioglitazone again NAFLD fibrosis score was calculated for every patients. Before and after NAFLD score was compared to assess change in severity of NAFLD in type II diabetes. Also NAFLD fibrosis score correlated with anthropometric parameters like BMI and biochemical parameters at time of starting of pioglitazone therapy and after 6 month of therapy. Data was analyzed using software Epiinfo version 7.2. Results were presented in the form of tables & graphs. Age, gender & various scores were compared in both groups. Mean values of scores was compared using unpaired T test. P value less than 0.05 was considered for significance

RESULTS

Table 1 Distribution of study subjects according to age and gender

Demographic Characteristic	Number (%)
Mean age	53.08 ± 6.94
Males	116 (77.33)
Females	34 (23.64)

Male: Female ratio of study subjects was 3.51:1. Mean age of study subjects was 53.08 ± 6.94



Study parameters before and after treatment were compared.

Table 2 Comparison of mean body mass index

BMI	Before treatment	After treatment	P Value
Mean ± SD	28.26 ± 5.63	29.86 ± 4.53	<0.01

Mean BMI both before and after treatment were in the obese category. Body mass index was more after treatment as compared to before treatment.

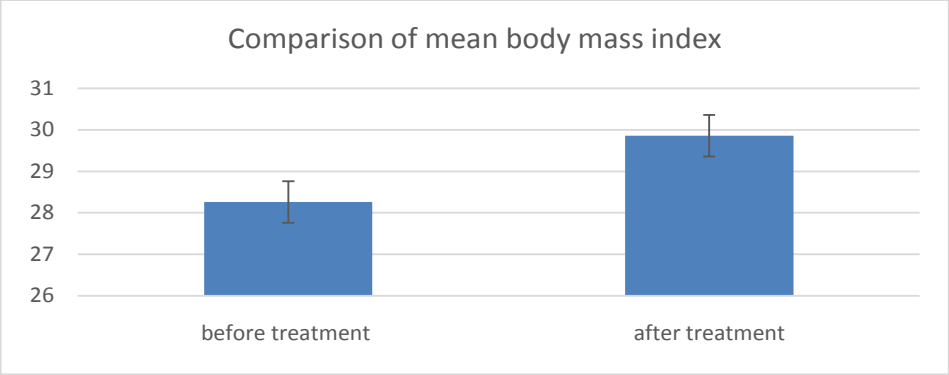


Table 3 Comparison of mean fasting blood glucose (FBS) level

FBS level	before treatment	after treatment	P Value
Mean ± SD	128.17 ± 20.42	104.75 ± 10.59	<0.01

Mean fasting blood glucose level was significantly decreased after treatment. (p<0.05)

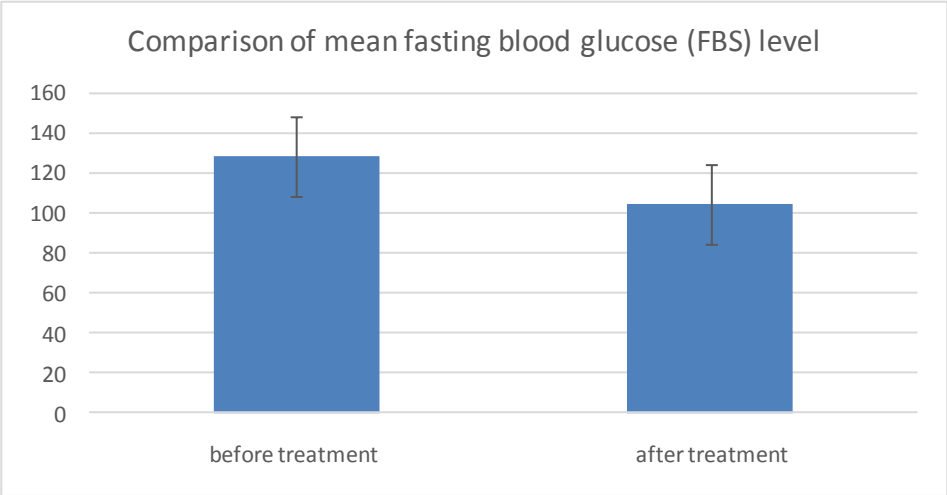
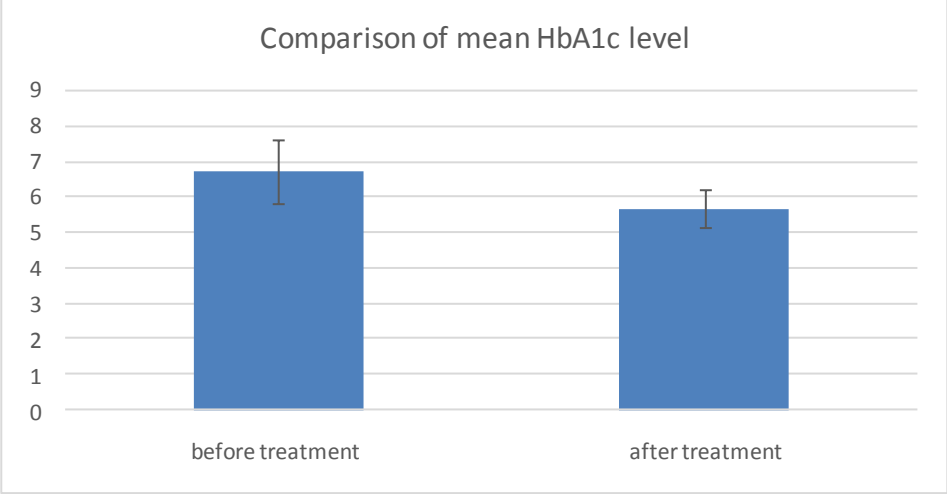


Table 4 Comparison of mean HbA1c level

HbA1c level	before treatment	after treatment	P Value
Mean ± SD	6.72 ± 0.90	5.67 ± 0.53	<0.01



Mean HbA1c was significantly decreased after treatment with pioglitazone. (p<0.05)

Table 5 Comparison of lipid profile level

Lipid profile	Before treatment Mean ± SD	After treatment Mean ± SD	P Value
Total cholesterol	192.98 ± 11.14	197.07 ± 29.97	0.23
LDL	121.23 ± 23.48	120.95 ± 9.75	0.81
HDL	40.16 ± 6.84	41.22 ± 8.60	0.23
Triglyceride	156.47 ± 22.28	156.37 ± 18.59	0.96

There was no significant difference in lipid profile after the treatment with pioglitazone. (p>0.05)

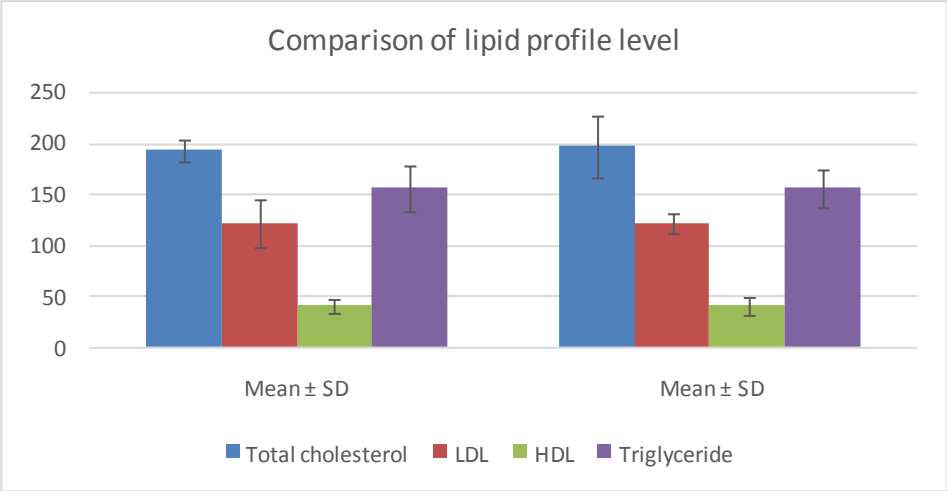


Table 6 Comparison of aspartate aminotransferase (AST)

AST	before treatment	after treatment	P Value
Mean ± SD	58.67 ± 5.48	34.88 ± 8.35	<0.01

Mean aspartate aminotransferase significantly decreased after treatment with pioglitazone.

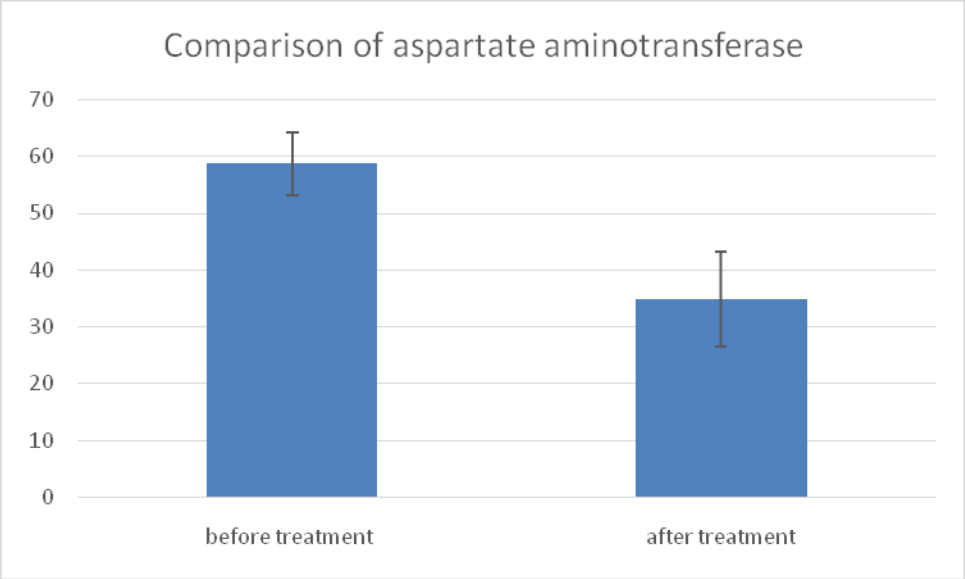


Table 7 Comparison of alanine aminotransferase (ALT)

ALT	before treatment	after treatment	P Value
Mean ± SD	60.36 ± 10.29	36.30 ± 4.73	<0.01

Mean alanine aminotransferase significantly decreased after treatment with pioglitazone.

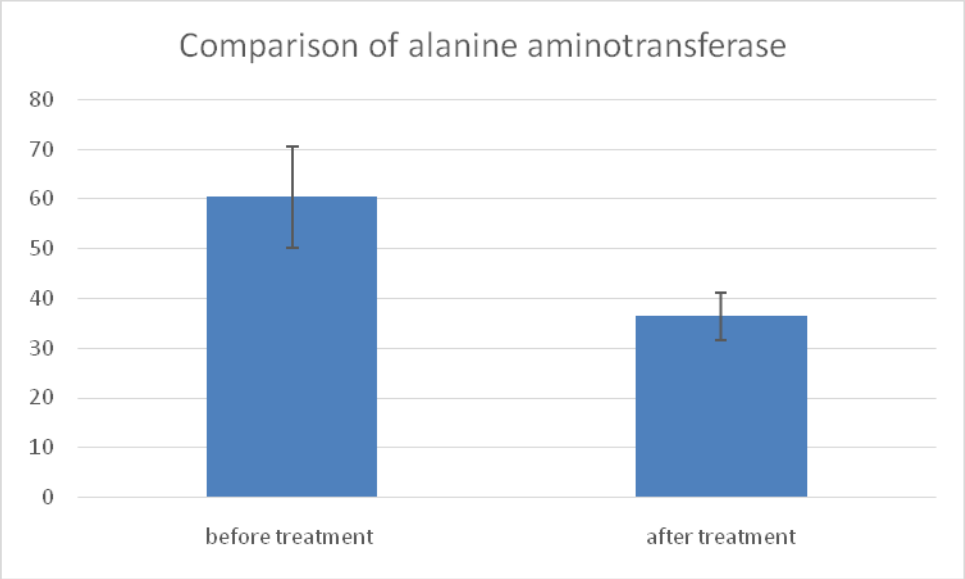


Table 8 Comparison of serum albumin

Serum Albumin	Before Treatment	After Treatment	P Value
Mean ± SD	3.55 ± 0.28	4.38 ± 0.25	<0.01

Mean serum albumin significantly increased after treatment with pioglitazone.

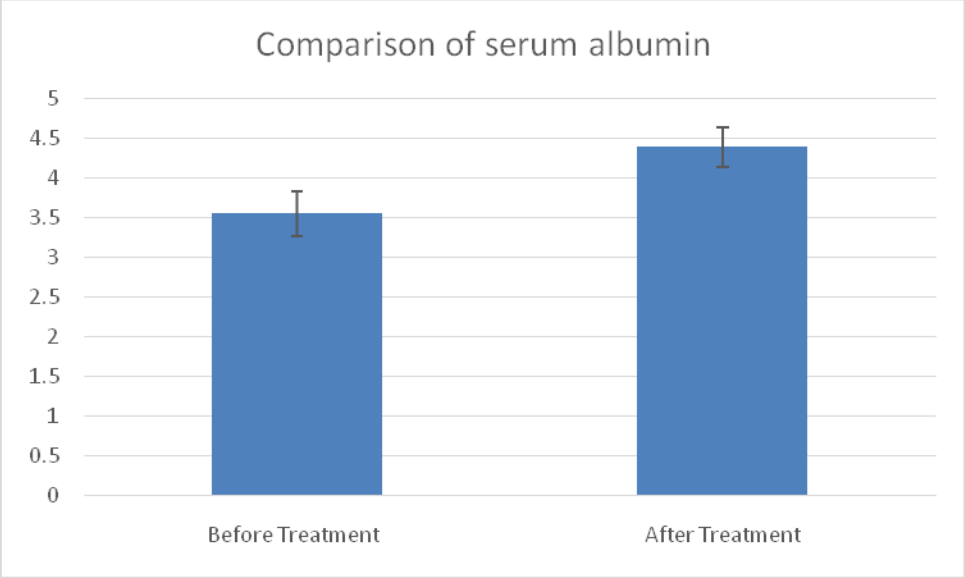


Table 9 Comparison of platelet count

platelet count	before treatment	after treatment	P Value
Mean ± SD	248651 ± 36706	301991 ± 59573	<0.01

Mean platelet count significantly increased after treatment with pioglitazone.

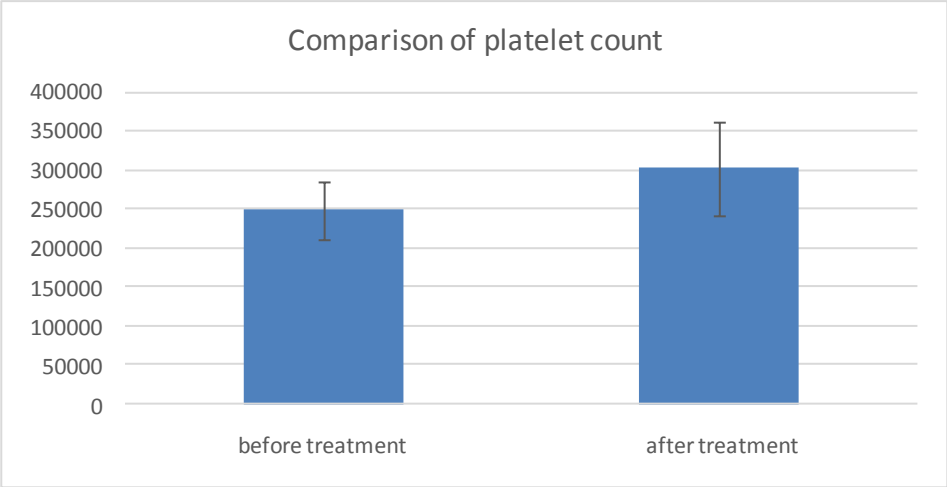


Table 10 Comparison of NAFLD Fibrosis Score (NFS) score

NFS score	before treatment	after treatment	P Value
Mean ± SD	-0.56 ± 0.21	-1.62 ± 0.30	<0.01

NAFLD Fibrosis Score significantly decreased after treatment with pioglitazone.

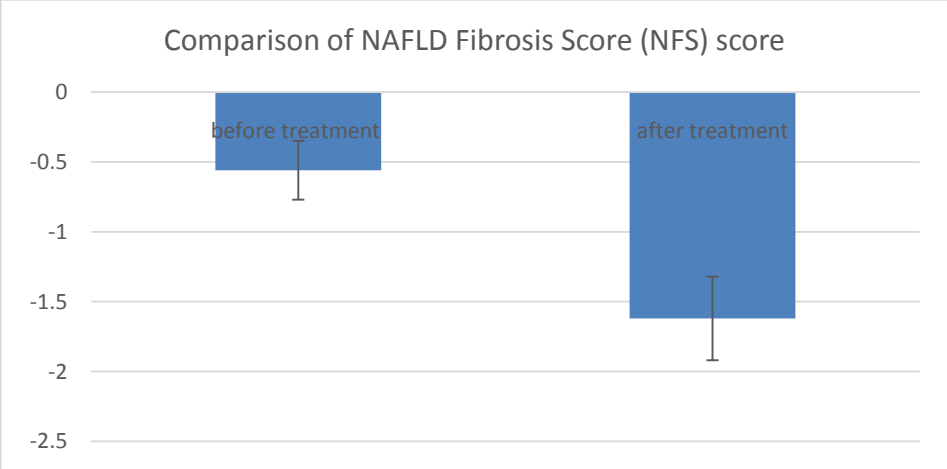


Table 11 Correlation between NAFLD fibrosis score with anthropometric and biochemical parameters after treatment

Parameter	NAFLD fibrosis score	
	Correlation coefficient	P value
BMI	-0.95	<0.01
Fasting blood glucose level	-0.89	<0.01
HbA1c	-0.88	<0.01
aspartate aminotransferase	-0.94	<0.01
alanine aminotransferase	-0.93	<0.01
Serum albumin	-0.93	<0.01
Platelet count	-0.97	<0.01

There was significant negative correlation between various an anthropometric and biochemical parameters and NAFLD fibrosis score. ($p<0.01$)

DISCUSSION-

Present study was Prospective Interventional study carried out in a tertiary care hospital from August 2016 to August 2018. Total 150 study subjects with NAFLD & type II diabetes were included in the study. Pioglitazone was given to all study participants for 6 months. NAFLD fibrosis score was calculated before start of treatment and after 6 month of treatment. At initiation of treatment and after 6 month of treatment severity of NAFLD was assessed with help of NAFLD fibrosis score. At end of pioglitazone therapy NAFLD fibrosis score was correlated with anthropometric & biochemical parameters.

In our study after treatment with pioglitazone therapy at the end of 6 months fasting blood sugar, HbA1c, aspartate transaminase, alanine transaminase and NAFLD fibrosis score was significantly decreased, while BMI, platelet count & serum albumin level was significantly increased. At the end of pioglitazone therapy there was no significant change in lipid profile. Significant decrease in NAFLD fibrosis score after 6 month of treatment with pioglitazone indicate that there was significant reduction in NAFLD in type II diabetes.

Renata Belfort (2006)(23) obtained similar finding in their comparative study in which they have compared pioglitazone therapy with placebo. They found that after treatment with pioglitazone therapy fasting blood sugar, HbA1c, aspartate transaminase, alanine transaminase and NAFLD fibrosis score was significantly decreased, while BMI, platelet count & serum albumin level was significantly increased ($p<0.01$). At the end of pioglitazone therapy there was no significant change in lipid profile ($p>0.05$).

Belfort R (2007)(23) in their comparative interventional study found that the administration of pioglitazone lead to metabolic and histologic improvement in subjects with nonalcoholic steatohepatitis. Larger controlled trials of longer duration are warranted to assess the long-term clinical benefit of pioglitazone.

Guruprasad P. Aithal (2008)(25) in their randomized placebo control trial found that compared with placebo, pioglitazone therapy was associated with an increase in weight (mean change, -0.55 vs -2.77 kg; P =.04) and a reduction in glucose (-0.4 vs -0.1 mmol/L; P=02), HbA1c (-0.16% vs -0.18%; P = .006), insulin C peptide level (-42 vs -78 pmol/L; P = .02), alanine aminotransferase level (-10.9 vs -36.2 u/L; P = .009), -glutamyl transferase level (-9.4 vs -41.2 u/L; P = .002), and ferritin (-11.3 vs -90.5 g/L; P = .01). Histologic features including hepatocellular injury (P = .005), Mallory–Denk bodies (P = .004), and fibrosis (P = .05) were reduced in patients treated with pioglitazone compared with those in the placebo group. At end they have concluded that Pioglitazone therapy over a 12-month period in nondiabetic subjects with NASH resulted in improvements in metabolic and histologic parameters, most notably liver injury and fibrosis. These finding in non-diabetic patients were exactly similar to our study finding.

Keith G. Tolman (2009)(33) in their comparator controlled study found that hepatic safety profile of pioglitazone similar to that of glibenclamide in long-term use in patients with poorly controlled type 2 diabetes. **Ajay Duseja (2010)**(35) in their review mentioned that for treatment of NAFLDweight loss, thiazolidinedione's (especially pioglitazone), and antioxidants have been most extensively evaluated. Weight loss was found to be safe and dose-dependently improved histological disease activity in NASH, but more than 50% of patients failed to achieve target weight loss. Thiazolidinedione's improved steatosis and inflammation but yielded significant weight gain.

R. Lomonaco et al. (2013)(36) in their review they have mentioned number of evidence which shows that in patients with NASH, pioglitazone reduce subclinical inflammation, improve adipose tissue and hepatic insulin sensitivity, and restore liver histology. Several relatively small RCTs have demonstrated the efficacy of TZDs in patients with steatohepatitis. They also mentioned that only study in patients with prediabetes or T2DM and NASH, pioglitazone (45 mg/day) significantly diminished insulin resistance at the level of the liver, adipose tissue and muscle and improved liver steatosis, necroinflammation and hepatocellular ballooning when compared with placebo.

K. Cusi (2016)(32) in their report they stated that more patients in the pioglitazone group showed improvement in their liver biopsies. In about half of the patients receiving pioglitazone, the liver biopsies no longer showed NASH. The pioglitazone group showed more improvement in fasting blood sugar levels, liver function tests, and triglyceride levels but had a weight gain of about 5.5 pounds compared with the placebo group. No patients developed bladder cancer, osteoporosis, or bone fractures due to osteoporosis.

Jonathan M. Hazlehurst (2016)(9) in their review mentioned one study which includes 63 participants with biopsy confirmed NASH were randomized to receive either rosiglitazone or placebo for 1 year. In that trial conclusion was steatosis improved, there was no improvement in fibrosis or in the NAFLD activity score. **Vasilios G. Athyros (2017)**(37) in their statement highlighted that first line pharmacological treatment for NASH pioglitazone, statins (high intensity, high dose) and ezetimibe alone or in combination although further specifically designed randomized trials are certainly needed.

Giovanni Musso (2017) (34) in their meta-analysis concluded that pioglitazone use improves advanced fibrosis in NASH, even in patients without diabetes. Whether this finding translates to improvement in risk for clinical outcomes requires further study. **Fernando**

Bril (2017) (38) in their review stated that patients with NASH and prediabetes or T2DM, the evidence appears to show that pioglitazone has the greatest treatment effect. It targets not only liver histology, but also the underlying metabolic disturbances, in particular insulin resistance. Of note, histological improvement after pioglitazone therapy is closely correlated with the reversal of adipose tissue insulin resistance and an increase in plasma adiponectin levels.In the long term, its metabolic and histologic benefits appear to persist over time, but they wane after treatment discontinuation.

CONCLUSION

In present study we found that:

- The administration of pioglitazone was led to metabolic improvement in type II diabetic subjects with NAFLD.
- NAFLD fibrosis score was very useful non-invasive tool to assess fibrosis in NAFLD in type II diabetic subjects

Larger controlled trials of longer duration are warranted to assess the long-term clinical benefit of pioglitazone.

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