ABSTRACT

PHASE I

Introduction: Non-alcoholic fatty liver disease (NAFLD) has received very little attention as a potent complication in type 2 diabetics. It is a condition of ectopic fat deposition in the hepatocytes in the absence of significant ethanol intake. It is the most common hepatic disease, yet it remains under-recognised. In the past two decades the prevalence of NAFLD has doubled whereas that of other chronic liver diseases has either stabilized or decreased. Asian Indians are at higher risk of developing NAFLD as they develop central obesity instead of general obesity and due to ethnic predisposition to type 2 diabetes makes the possibility of having NAFLD very high in the Indian population. Metabolic syndrome (MS) and NAFLD share the same pathophysiologic soil of insulin resistance and hyperinsulinemia, thus NAFLD denotes hepatic expression of the MS. The data on type 2 diabetics with NAFLD and association with MS has been lacking in the Indian context. While social health assessment still awaits inclusion as a part of standard care, quality of life of a type 2 diabetic NAFLD patient needs to be brought to light.

Objectives: To map the prevalence of NAFLD among type 2 diabetes patients, assess their cardio-metabolic, dietary profile, physical activity profile, prevalence of metabolic syndrome, arrive at the predictor variables for NAFLD in type 2 diabetics and determine the quality of life of type 2 diabetes patients with NAFLD.

Methods and Materials: Type 2 diabetics (N=105) were evaluated for anthropometry, blood pressure, diet, physical activity and a fasting blood sample was obtained to assess complete blood count, lipid, renal, thyroid, hepatic profile, hs-CRP, HbA1c. Two were diagnosed hepatitis B positive and another hepatitis C positive and were excluded from the study. Seven others declined to appear for abdominal ultrasound. Therefore, 95 type 2 diabetics underwent abdominal ultrasonography for diagnosis of NAFLD.

Results I (A): The prevalence of ultrasound diagnosed NAFLD was 77.9% (CI 69.4-86.4); 80.8% among females and 74.4% among males. Grade 2 steatosis was most prevalent (61.1%), followed by grade 1 steatosis (10.5%) and grade 3 steatosis was

least prevalent (6.3%). NAFLD subjects had significantly higher BMI (28.4 vs. 25.3kg/m^2, P 0.007), WC (98.9 vs. 89.2cm, 0.0006) and WSR (0.62 vs. 0.55, P 0.001). NAFLD subjects had significantly lower intake of crude fibre (5.4 vs. 6.7g, P 0.0031) and vitamin A (114.1 vs. 175.2 µg, P 0.025). The NAFLD subjects consumed significantly lower proportion of protein in their diet compared to normal liver subjects (10.9% vs. 12.6%, P 2.84E). The NAFLD subjects had significantly higher non HDL-C (135.9 vs. 119.4mg/dl, P 0.03), hs-CRP (4.83 vs. 2.74mg/l, P 0.017), liver span (168.2 vs. 157.3mm, P 0.019) and number of features of MS (3.3 vs. 2.7, P 0.036) and had significantly lower physical activity level (703.8 vs. 1200.5total METminutes/week, P 0.017) than those with a normal liver.

Consumption of cottonseed oil (45.9% vs. 14.3%, P 0.008, OR: 5.1, CI: 1.26-23.92), presence of obesity (74.3% vs. 42.9%, P 0.006, OR: 3.86, CI: 1.26-11.99), abdominal obesity (87.8% vs. 57.1%, P 0.0016, OR: 5.42, CI: 1.57-19.09), AVI >16 (79.7% vs. 47.6%, P 0.003, OR: 4.33, CI: 1.38- 13.74), hs-CRP >3mg/l (58.1% vs. 28.5%, P 0.017, OR: 3.47, CI: 1.09-11.41), AIP >0.21 (82.4% vs. 57.14%, P 0.015, OR 3.52, CI: 1.09-11.46), GGT >35U/L (28.3% vs. 4.7%, P 0.036, OR: 7.92, CI: 1.01-168.34), liver span >160mm (64.9%, vs. 38.1%, P 0.028, OR: 3, 1-9.22) and presence of MS (72.9% vs. 33.3%, P 0.008, OR: 5.4, CI: 1.71-17.54) were identified as risk factors for NAFLD in type 2 diabetics. The presence of MS was the strongest predictor of NAFLD in type 2 diabetics (P 0.002, OR 5.4, CI: 1.9-15.3). With increasing prevalence of the features of MS, the prevalence of NAFLD increased and so did the grade of hepatic steatosis (P 0.023). Those with three (P 0.048) and four features of MS (P 0.006) had significantly higher prevalence of NAFLD and grade of hepatic steatosis compared to those with two features of MS.

Conclusions: The prevalence of NAFLD was alarmingly high in the type 2 diabetics. NAFLD subjects had an unfavourable cardio-metabolic profile, lower physical activity status and a close association with metabolic syndrome. Subjects with type 2 diabetes should be assessed annually for the presence of metabolic syndrome. Should metabolic syndrome be present, the subjects should be assessed for GGT elevation. If GGT levels are >35U/L, the subjects should then only be screened by NAFLD through ultrasonography.

Result (I B): Female NAFLD subjects had significantly lower scores on the following domains than the males; role limitation due to physical health (3.8 vs. 4.2, P 149E), physical endurance (3.3 vs. 4.09, P 1.62E), symptom botherness (4 vs. 4.4, P 1.72E), general health (3.2 vs. 3.8, P 2.12E), treatment satisfaction (3.1 vs. 3.8, P 3.09E), financial worries (3.4 vs. 4.07, P 2.91E) and emotional/mental health (3.8 vs. 4.3, P 1.74E). Gender wise as well as stage of hepatic steatosis wise, diet satisfaction was the lowest scoring domain in quality of life assessment. The grade 3 hepatic steatosis subjects had significantly lower scores than the grade 1 hepatic steatosis subjects (P 0.0024), (P 0.0005), (P 0.002) and grade 2 hepatic steatosis subjects (P 0.0024), (P 0.0001), (P 0.0067) in role limitation due to physical health, physical endurance and treatment satisfaction, respectively.

Conclusions: The quality of life of female NAFLD subjects was more compromised than the male NAFLD subjects. Physical health, endurance profile and diet profile were the least scoring domains. However, they are modifiable risk factors that can be modulated to enhance self care management of the disease. It also calls for inculcation of quality of life assessment in the routine standard care protocol in order to impart holistic treatment.

PHASE II

Introduction: The current treatment and management modalities for NAFLD revolve around correcting the underlying metabolic abnormalities associated with NAFLD and discontinuing hepatotoxic drugs, if any, as there are no evidence based guidelines. However, NAFLD can be reversed if the underlying metabolic aberrations are corrected through lifestyle modification. Therefore, lifestyle interventions are considered as the cornerstone of management of NAFLD and propagated as the first line therapy for the treatment of NAFLD. Thus, delving into the dietary and physical activity profiles of the NAFLD patients may hold scope for introducing lifestyle modification.

Objectives: To assess the knowledge attitude and practices of type 2 diabetes patients with NAFLD and determine the impact of lifestyle modification counselling in the management of NAFLD and impact on knowledge attitude and practices.

Methods and Materials: Sixty type 2 diabetic NAFLD subjects were enrolled from the previous phase of the study based on voluntary consent and were randomly allocated into either of the two groups; intervention arm to receive nutrition counselling propagating lifestyle modification in addition to standard care and control group to be on standard care only. The subjects were evaluated for knowledge attitude and practice regarding type 2 diabetes and NAFLD. They were provided nutrition counselling once in a month for a period of four months and were provided with a booklet on the management of NAFLD in type 2 diabetes to be used as a ready reckoner. Anthropometric indices, blood pressure, diet and physical activity data was collected monthly and blood profile measuring hepatic, renal, thyroid, lipid, hs-CRP and HbA1c, along with abdominal ultrasound was carried out pre and post 4 months intervention to assess the impact of nutrition counselling.

Result (II B): The KAP score improved from 16.6 to 44.03 (P 1.8E) with the nutrition counselling intervention and among controls improved from 16.5 to 23.7 (P 1.05E) because of which the intervention subjects scored higher from controls (44.03 vs. 23.7, P 8.72E). The intervention reduced the prevalence of low KAP score (93.3% to nil, P 0.000), increased the prevalence of average KAP score (6.6% to 80%, P 0.000) and of good KAP score (nil to 20%, P 0.023), and therefore the intervention arm was better than the controls in all the three categories (P 0.000), (P 0.0001), (P 0.023), as controls decreased on low score from 96.6% to 70% (P 0.005), increased average score from 3.3% to 30% (P 0.005) and was nil in good KAP score prevalence.

Results II (C): The prevalence of NAFLD declined significantly with the intervention (100% to 63.3%, P 0.0002) and became lower from controls (63.3% vs. 96.7%, P 0.0013), along with reduction in severity of steatosis (1.86 to 1.2, P 0.00016) which was significantly lower from controls (1.2 vs. 1.93, P 0.0003). In controls only 3.3% NAFLD subjects reversed to normal liver. The intervention subjects attained significantly lower weight than the controls (P 0.05, 65.8kg vs. 72.1kg). The soluble fibre intake increased significantly with the intervention (P 0.041). The proportion of energy coming from fat was significantly lower in the intervention arm compared to the controls at baseline (32.8% vs. 35.4%, P 0.011) and

at 1st month (29.05% vs. 33.1%, P 0.028). SBP (145.2 to 128.1mmHg, P 3.23E), ferritin (67.1ng/ml to 50.7ng/ml, P 0.025), triglycerides (138.7mg/dl to 121.5mg/dl, P 0.031), hs-CRP (4.6mg/l to 3.4mg/l, P 0.024), alkaline phosphatase (90.5U/L to 81.2U/L, P 0.005), GGT (32.4U/L to 26.3U/L, P 0.0016) and liver span (173.5mm to 166.4mm, P 0.037) declined significantly with the intervention. SGPT reduced significantly (26.05U/L to 20.7U/L, P 0.03) after the intervention and was also lower from the controls (20.7U/L vs. 27.8U/L, P 0.014). The total METminutes/week increased non-significantly during the intervention period in the experimental arm even though the HDL-C increased (47.2mg/dl to 52.2mg/dl, P 3.6E). HDL-C of the intervention arm subjects was also significantly higher than the controls (52.2mg/dl vs. 46.7mg/dl, P 0.049). The prevalence of MS came down from 76.7% to 53.3% in the experimental arm as the number of features of MS significantly reduced from 3.66 to 3.03 (P 0.0008).

Prevalence of NAFLD reduced significantly from 100% to 61.1% in subjects who lost ≥7% weight (P 0.007), with liver span reduction from 179.1 to 167.3mm (P 0.004). Prevalence of MS reduced from 77.7% to 44.4% (P 0.043) as the number of features reduced from 3.6 to 2.72 (P 0.0006). Subjects with ≥7% weight loss had more profound reduction in BMI (9.9% vs. 4.8%), WC (6.4% vs. 3.4%), WSR (6.3% vs. 3.2%) and SBP (12.7% vs. 10.5%). They also had a significant increase in soluble fibre intake (P 0.017) and their proportion of protein intake increased significantly (P 0.034). These subjects had significantly lower frequencies of eating out than the subjects who lost <7% weight. Prevalence of hypertriglyceridemia became significantly lower in subjects who lost ≥7% weight compared to those who lost <7% weight (16.7% vs. 58.3%, P 0.045) and prevalence of AIP>0.21 reduced from 94.4% to 61.1% in subjects with ≥7% weight loss (P 0.040). GGT reduced significantly from 28 to 22.4U/L in subjects with ≥7% weight loss (P 0.007) and also became significantly lower from subjects who had <7% weight loss (22.4 vs. 32.3U/L, P 0.033). These subjects had a significant increase in total METminutes/week (P 0.012) and had better profile than subjects who lost <7% weight (P 0.032) as they increased on their HDL-C (P 0.0007).

Conclusions: Nutrition counselling propagating lifestyle modification was able to bring about significant changes in the KAP score of type 2 diabetic NAFLD subjects

that led to an improvement in all the dimensions of health ranging from anthropometric, biochemical and imaging of the liver. It corroborates that nutrition counselling can be a practical approach to empower the patients with knowledge that can lead to possible changes in attitudes and practices as self management improves. NAFLD needs attention as a serious metabolic co-morbidity in type 2 diabetics that calls for not only treatment but prevention of NAFLD by means of nutrition education. In the absence of evidence based guidelines, nutrition counselling propagating lifestyle modification holds the key for reversal of NAFLD.

PHASE III

Introduction: Tinospora cordifolia belongs to the family *Menispermaceae* and is a rich source of alkaloid and terpenes. The tinospora cordifolia stem is approved for medicinal usage. Pre-clinical studies have established the efficacy of tinospora cordifolia stem as an anti-dyslipidemic and anti-diabetic agent. However, there are no clinical studies to corroborate the same. With a host of phytochemical properties present in the stem, the plant may hold potential to manage dyslipidemia as well as dysglycemia in patients with type 2 diabetes.

Objective: To analyse the qualitative phytochemical profile of tinospora cordifolia stem and assess its impact in the management of diabetic dyslipidemia.

Methods and Materials: Mature stem of tinospora cordifolia was subjected to qualitative phytochemical screening. For the supplementation, type 2 diabetics with dyslipidemia on oral hypoglycemic agents and statin were enrolled from a clinic. They were evaluated for disease and drug profile, anthropometric, blood pressure, dietary and physical activity assessment. A fasting blood sample was obtained to estimate lipid, renal, thyroid and glycemic profile. The subjects were randomized in either of the two arms; supplementation group (n=29) that received 250mg of encapsulated mature stem of tinospora cordifolia pre meal twice a day along with statin and control group (n=30) which was only on statin for a period of 60 days. After 60 days all the parameters were re-assessed to analyse the impact of the intervention.

Results: Presence of alkaloids, tannins, flavonoids, terpenoids and cardiac glycosides was observed in tinospora cordifolia stem sample. Majority of the subjects were in the 50-60 years age bracket and had a similar duration of diabetes and drug profile. Tinospora cordifolia supplementation brought about a significant decline in WC (94.7 to 94.2cm, P 0.004), WSR (0.594 to 0.591, P 0.004) and SBP (132.6 to 127.1mmHg, P 0.0017) vs. significant decline in SBP (134.5 to 130.1mmHg, P 0.0013) in controls. The physical activity and dietary profiles were alike for both groups. Uric acid declined significantly with the intervention (5.2mg/dl to 4.6 mg/dl, P 0.007) and was also lower from the controls (4.6mg/dl vs. 5.7 mg/dl, P 0.0018). Hs-CRP declined significantly from 4.6mg/l to 2.8mg/l (P 0.0007) and reduced the prevalence of hs-CRP >3mg/l (65.5% to 37.9%, P 0.037) with intervention. Supplementation led to significant reductions in TC (208.3 to 178mg/dl, P 0.0008), LDL-C (122.4 to 105.8mg/dl, P 0.0028), triglycerides (146.1 to 124.3mg/dl, P 0.036) and VLDL-C (28.9 to 23.6mg/dl, P 0.003). The controls too had significant reductions, but of lesser intensity. The mean dyslipidemic features declined by 28.6% (2.27 to 1.62, P 0.0036) and by 19.4% (2.06 to 1.66, P 0.020) among controls. Reduction in HbA1c was more evident in the supplementation group (7.7% to 7.5%, P 0.09) than the control group (from 7.9% to 7.81%, P 0.52). The prevalence of MS declined from 68.9% to 55.17% as the number of features of MS reduced (3.2 to 2.86, P 0.059) with the intervention.

Conclusions: The mature stem of tinospora cordifolia contained tannins, alkaloids, flavonoids, terpenoids and cardiac glycosides. Supplementation with 500mg pure extract tinospora cordifolia stem in diabetic dyslipidemia for a period of 60 days had a significant anti-inflammatory and anti-dyslipidemic effect. It led to more evident changes, though of non-significant nature, in glycemic profile and MS, than the controls.