JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2023/30616

RESEARCH ARTICLE

The Effect of *Sira Vedha* (Phlebotomy) at Right Elbow Joint in the Management of Non-alcoholic Fatty Liver Disease - Randomized Controlled Clinical Trial

Gaurav Sawarkar[1*], Priti Desai[2] and Punam Sawarkar[3]

[1]Department of Rachana Sharir, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Datta Meghe Institute of Higher Education and Research (Deemed to be University), Wardha - 442001, Maharashtra, India; drsawarkar.gaurav@gmail.com

[2]Department of Rachana Sharir, Sardar Patel Ayurvedic College And Hospital, Sardar Patel University, Dongariya - 481331, Balaghat, Madhya Pradesh, India

[3]Department of Panchakarma, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Datta Meghe Institute of Higher Education and Research (Deemed to be University), Wardha - 442001, Maharashtra, India

Abstract

Introduction: *Sushruta* stated that *Sira Vedha* (phlebotomy) at Right Elbow Joint was used as a cure for Liver Diseases. Modern science is likewise on the same page as phlebotomy treatment for some liver illnesses, although the specific location for phlebotomy has not been specified. Similarly, there is diversity in the amount of blood extracted and the time length, with each research study recommending a different amount to be removed and the gap between two sessions. Both of the previously listed things are mentioned in the context of *Ayurveda*. Nonetheless, due to a lack of clinical proof, it cannot be practiced reliably in normal exercise. As a result, clinical proof for liver illnesses is required to confirm Sushruta's theory. **Methodology:** 111 eligible patients with Non-Alcoholic Fatty Liver Disease (NAFLD) Grade I/II were randomly assigned to the control (group A) or trial (group B) groups in the pilot research (group B). After obtaining written informed permission, blood samples from each patient were collected for LFT and lipid profile testing. The patients in Group A were then just counseled to make dietary changes. Group B patients underwent dietary changes as well as *Sira Vedha* (phlebotomy) with 65 cc blood three times in 15 days. Checked hemoglobin before each phlebotomy for safety considerations before discontinuing the patient. The blood sample was taken before enrollment in the study and after the final follow-up, which was on the 60th day. **Results:** The serum results from the first and last follow-ups were compared. This study found that group B considerably improved in liver enzymes and lipid profile (P<0.050) when compared to group A. As a result, we may infer that phlebotomy can enhance liver enzymes and lipid profiles in NAFLD patients.

Keywords: Blood Letting, Liver Disease, NAFLD, Phlebotomy

1. Introduction

Siravedha is associated with phlebotomy from a modern perspective. Phlebotomy has been used in healthcare for millennia and is still one of the most popular invasive procedures. The process of withdrawing blood from the circulatory system by an incision or puncture to acquire a sample for examination, diagnosis, and management is known as phlebotomy[1].

*Author for correspondence

Article Received on: 05.07.2022 Revised on: 05.05.2023 Accepted on: 08.07.2023

Non-alcoholic fatty liver disease is the most frequent of the many liver ailments mentioned above, and its incidence is rising by the day. Phlebotomy is successful in the treatment of Non-Alcoholic Fatty Liver Disease (NAFLD) by reducing liver cell damage and improving liver enzymes[2].

According to a recent scientific study, blood volume withdrawn during bloodletting ranged from 250 - 500 ml. Bloodlettings were spaced out over a week to a month apart. As predicted, venesection treatment did not go down well with the patients in such an amount of blood. According to Piperno, *et al.,* many venesected individuals suffered weariness and moderate malaise[3]. In clinical studies conducted in *Ayurveda*, blood withdrawal was around 27 - 100 ml with 3 to 4 settings in one or two months[4-8]. In *Ayurveda* context bloodletting suggested with an interval of 15 days as per condition[9].

The present study is an effort to generate collateral evidence supporting *Sushruta's* remark of *Siravedha* (Phlebotomy) at right elbow joint in liver diseases (NAFLD).

NAFLD is one of the commonest conditions associated with the Liver. The prevalence of NAFLD among the general population in India ranges from 9 - 53 % (De A, Duseja A, 2021). However, no study was conducted in an Indian setting on *Sira Vedha* (Phlebotomy) (phlebotomy) for liver disease/NAFLD. Clinical studies were conducted mainly on *Gridhasi* (Sciatica), *Vicharchika* (Eczema)[2], foot ulcers[1]. The volume of blood withdrawn in said studies ranges from 27 - 100 ml with 3 to 4 sittings. However, one could not find a single clinical study related to the use of *Sira Vedha* (Phlebotomy) in NAFLD. Considering modern scientific research studies, the amount of blood withdrawn during bloodletting ranges from 250 - 500 ml. The intervals between bloodlettings ranged from one week to one month. Taking into account all of the above information, it was thought to generate clinical evidence for the use of *Siravedha* at right elbow joint in Non-Alcoholic Fatty Liver Disease along with diet and exercise (lifestyle modification).

2. Research Design

Type of Study: Interventional
Study design: Randomized Controlled Single-Blind Comparative Three Armed Clinical Trial.
Sample Size - 111
Group A (Control Group): 57
Group B (Interventional Group): 54
Screening parameters – Clotting time, Bleeding time, Haemoglobin, Liver Function Test, Lipid profile, USG.
Assessment parameters - Liver Function Test, Lipid profile, Weight, B.M.

3. Methodology

Source of Data: Subjects were collected from OPD and IPD, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H).
Type of Study: Interventional.
Study design: Randomized Controlled Single-Blind Comparative Three Armed Clinical Trial.
Sampling procedure: Simple Randomization through computer generated table.
Grouping:
Group A (Control Group): Diet and Moderate Exercise (Figure 1) (Table 1).
Group B (Interventional Group): *Sira Vedha* (Phlebotomy) at right elbow joint with lifestyle modification (Diet and Moderate Exercise) (Figure 1) (Table 1).
Group C (External Control): Phlebotomy with lifestyle modification[10,11] (Figure 1) (Table 1).
IEC approval: DMIMS (DU)/IEC/2018-19/7351
CTRI registration: CTRI/2020/04/024783
Population: Diagnosed Non-Alcoholic Fatty Liver Disease (NAFLD) Grade 1 and 2.

3.1 Inclusion Criteria

Patients who are willing to participate in the study by giving written informed consent, diagnosed Non - Alcoholic Fatty Liver Disease (Grade I and Grade II fatty liver), patients between age group of 20 to 50 years, irrespective of sex, patients of NAFLD fulfilling Screening parameters as clotting time - 8 -15 min, bleeding time- 2-7 min, hemoglobin - ≥ 11 g/dL (Male), ≥ 10 g/dL (Female), AST (SGOT) - ≥ 40 units per liter of serum, ALT (SGPT) - ≥ 56 units per liter of serum, total Cholesterol - ≥ 200 mg/dL, LDL - ≥ 130 mg/Dl, Triglycerides - ≥ 150 mg/dL, USG-NAFLD Grade-I/Grade-II.

3.2 Exclusion Criteria

The study excluded patients with patients contraindicated for Sirvedha (Sushrut Sharir Sthan 8/3), carcinoma of the liver, liver cirrhosis, HIV positive, HBsAg positive, terminal illness, pregnant and lactating lady, accidental cases, all types of anemias, hemophilia, thalassemia, case of poisoning, alcoholic liver diseases, eczema over right elbow joint, skin diseases/allergies, and injury at the right elbow joint.

3.3 Data Collection Tools and Process

Patients fulfilling the inclusion criteria and willing to give structured written consent were enrolled for the study. The selected Patients were informed about the study, possible outcome, side effects of intervention, if any, and right to withdraw. The selected patients were then assigned to control and interventional groups randomly using the lottery method by a person other than the researcher (Figure 1).

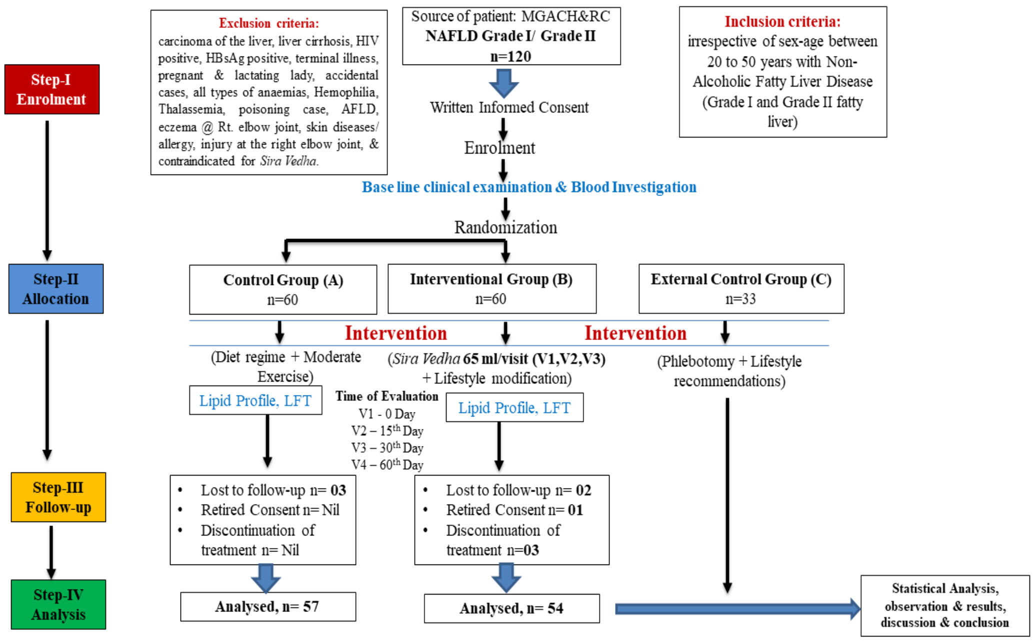

Figure 1. Consort diagram.

Table 1. Intervention and investigation plan

	Group A (Control Group)		**Group B (Study Group)**	
Sample size	57		54	
Intervention	lifestyle modification with a diet regime		*Sira Vedha* (Phlebotomy) at Right Elbow Joint + lifestyle modification with diet regime	
Bloodletting Quantity	-		65 ml per visit	
Duration	Day 0	Investigations before intervention	Day 0	Investigations before intervention
	Day 1	Instructions and counseling for diet and exercise	Day 1	1st Visit- *Sira Vedha* (Phlebotomy) + Instructions and counseling for diet and exercise
	Day 15	1st Visit Weight and BMI assessment	Day 15	2nd Visit- *Sira Vedha* (Phlebotomy) + Instructions and counseling for diet and exercise, Weight and BMI assessment
	Day 30	2nd Visit Weight and BMI assessment	Day 30	3rd Visit- *Sira Vedha* (Phlebotomy) + Instructions and counseling for diet and exercise, Weight and BMI assessment
	Day 31	Investigations	Day 31	Investigations after completion of the intervention
Follow up	60th day	Follow up (Post-intervention investigations)	60th day	Follow up (Post-intervention investigations)
Total duration		120 days		120 days

3.4 Lifestyle Modification and Diet Regime

3.4.1 Recommended Diet

Nuts/Seeds/Legumes, whole grain-rich fiber, olive oil, fruits, vegetables, and home-cooked meals were preferable. In the case of non-vegetarian- fish and seafood and a low intake of red meat in a limited manner (once or twice a month)[12-14].

3.4.2 Non-recommended Diet

Ultra-processed food, saturated fats and cholesterol, processed meat, sweets, high fat/ sugared dairy products, sugar-sweetened beverages, alcoholic beverages.

3.4.3 Recommended Physical Activity

Yoga and *Pranayam* minimum 60 minutes in the week (5 to 10 min per day) [Moore A, *et al.*, 2012], 150 minutes of weekly (20 - 25 min per day) accumulated moderate-intensity exercise[15].

4. Observation and Results

All data were compared using the paired and unpaired student's t-test for all predicted results as mean + Standard Deviation (SD). When $P<0.05$, the difference is deemed significant. SPSS (Statistics Package for Social Science) for Windows statistical software version 17.0 was used for statistical analysis, while Microsoft Excel was utilized to make graphs, tables, and other graphics. In the present research study, a total of 120 patients were recruited, 111 patients have completed the study, and 09 patients dropped out due to the Covid-19 pandemic and personal issues. Male patients were 21 and 19, whereas female patients were 36 and 37 in the Control group (A) and Interventional group (B). The mean age of females was observed as 46.95 ± 2.67 and 46.94 ± 2.41, while the mean age of male patients was seen as 43.52 ± 4.98 and 42.53 ± 3.75 in Control Group (A) and Interventional Group (B).

The Table 2 demonstrated percentage change in SGOT, before and after in control, external control, and

Table 2. SGOT (Serum Glutamic Oxaloacetic Transaminase)/AST (Aspartate Aminotransferase)- Mean, SD, Range of values – 95% confidence interval and percentage change

		Control	External Control	Intervention
Mean and SD	Before	47.59 ± 7.11	47.0 ± 20.6	48.57 ± 6.74
	After	41.63 ± 6.54	38.8 ± 14.2	25.29 ± 5.93
Range of values – 95 % confidence interval	Before	45.71 to 49.48	46.65 to 69.54	46.73 to 50.41
	After	39.89 to 43.37	35.8 to 57.99	23.67 to 26.91
Percentage Change	Lower	12.73 %	14.82 %	49.35 %
	Upper	12.35 %	19.34 %	46.62 %

Table 3. Serum glutamic pyruvic transaminase (SGPT)/ALT (Alanine transaminase) Mean, SD, Range of values – 95% confidence interval and percentage change

		Control	External Control	Intervention
Mean and SD	Before	69.08 ± 10.69	63.5 ± 30.6	70.55 ± 10.71
	After	61.74 ± 9.18	49.7 ± 18.2	36.24 ± 11.16
Range of values – 95% confidence interval	Before	66.25 - 71.93	61.54 - 98.46	67.63 - 73.48
	After	59.51 - 64.39	45.44 - 82.35	33.19 - 39.28
Percentage Change	Lower	10.17 %	17.70 %	50.92 %
	Upper	10.48 %	24.54 %	46.54 %

interventional group. Before respective intervention in all groups, the mean value was almost the same; it was the same for SD except in the external control group. On comparing the range of 95% confidence interval values, it was reflected that intervention group changes were observed as highly significant, i.e., percentage change lower limit- 49.35% and upper limit - 46.62%.

The Table 3 demonstrated percentage change in SGPT, before and after in control, external control, and interventional group. Before respective intervention in all groups, the mean value was nearly the same. Similarly, SD values were observed equally except in the external control group. After comparing the SGPT range of values 95% confidence interval, it was found that intervention group changes were detected as highly significant, i.e., percentage change lower limit - 50.92% and upper limit - 46.54%.

The Table 4 shows the percentage change in Serum Total Cholesterol before and after in the control, external control, and interventional group. Before respective intervention in all groups, the mean value was approximately the same except external control group. However, there was a difference in mean values after intervention and a difference in SD. After analyzing the range of 95% confidence interval values, it was reflected that intervention group changes were highly significant, i.e., percentage change lower limit - 38.42% and upper limit - 38.92%.

The Table 5 demonstrates before and after values of mean - SD and range of values – 95% confidence interval. There was no change observed in external control. On the contrary, the interventional group shows extremely significant changes, i.e., percentage change lower limit - 56.87% and upper limit - 53.71%.

The Table 6 denotes before and after values of mean-SD and range of values – 95% confidence interval. There were minor changes observed in external control at 6.46%. On the other hand, the interventional group shows significant changes, i.e., percentage change lower limit- 31.77% and upper limit - 32.08%.

Table 4. Serum Total Cholesterol- Mean, SD, Range of values – 95% confidence interval and percentage change

		Control	External Control	Intervention
Mean and SD	Before	243.42 ± 21.31	181.47 ± 46.33	244.41 ± 21.59
	After	217.71 ± 17.42	177.61 ± 46.33	149.81 ± 10.71
Range of values – 95% confidence interval	Before	237.77 - 249.08	164.8 - 198.2	238.52 - 250.3
	After	213.09 - 222.34	160.9 - 194.3	146.89 - 152.73
Percentage Change	Lower	10.38 %	2.34 %	38.42 %
	Upper	10.74 %	1.95 %	38.92 %

Table 5. High-Density Lipoprotein (HDL)

		Control	External Control	Intervention
Mean and SD	Before	34.42 ± 3.86	38.61 ± 11.58	34.5 ± 38.9
	After	42.07 ± 3.99	38.61 ± 11.58	53 ± 4.08
Range of values – 95 % confidence interval	Before	33.40 - 35.45	34.43 - 42.78	33.43 - 35.56
	After	41.01 - 43.13	34.43 - 42.78	52.44 - 54.66
Percentage Change	Lower	22.78 %	0 %	56.87 %
	Upper	21.66 %	0 %	53.71 %

Table 6. Serum Triglyceride - Mean, SD, Range of values – 95% confidence interval and percentage change

		Control	External Control	Intervention
Mean and SD	Before	205.21 ± 56.81	150.44 ± 53.10	209 ± 54.33
	After	158.28 ± 46.25	150.44 ± 79.65	142.33 ± 35.78
Range of values – 95% confidence interval	Before	190.14 - 220.29	131.3 - 169.6	194.27 - 223.94
	After	146.01 - 170.55	121.7 - 179.2	132.56 - 152.1
Percentage Change	Lower	23.21 %	7.29 %	31.77 %
	Upper	22.58 %	5.64 %	32.08 %

5. Discussion

5.1 Age and Sex

All patients recruited in the study were from 20 - 50 years old. In the present study, the age difference is observed in male and female patients in both groups. The 111 patients (40 males, 71 females) were recruited for the present study. The mean age of females was observed as 46.95 ± 2.72 and 46.94 ± 2.41, while the mean age of male patients was 43.52 ± 4.98 and 42.53 ± 3.75 in Group A and B, respectively. A maximum number of patients belonged to the fourth decade - 91.89%. Similar conclusions were found in a study by Dhumal, *et al.,* and Khanal, *et al.,* where both studies showed that the largest percentage of patients with non-alcoholic fatty liver belonged to the fourth to fifth decade age group[16,17]. It may be due to less engagement in physical activity in a given age group. Most of the metabolic disorders like diabetes, hypertension, and NAFLD are possibly a consequence of the lifestyle in this age group of the patients.

On the other hand, some studies reported that the prevalence of NAFLD was higher in men below forty years. In women over sixty years, these differences may be because of sex hormones[18]. In this study, Male to female ratio was 4:7. There is a debate about gender and NAFLD where some studies alleged that various gender-specific mechanisms, the role of sex hormones, and variations in lifestyles and physiology influence the prevalence of NAFLD. Different studies reported that NAFLD is more often found in men than women. But, at the same time, some studies imply the disease NAFLD is generally more common in women. Summart U., *et al.,* concluded that NAFLD is more common in women than men, stating that the age group belongs to the post-menopausal period[19]. Concerning the age of females having NAFLD, Ahuja, *et al.,* stated that the Indian women's perimenopausal age is about 44.69 ± 3.79 years, and the mean menopausal age is 45.59 ± 5.59 years[20]. These findings of various studies are similar to the present study, as the prevalence of NAFLD in women of the perimenopausal age is higher. The association between age and gender may be because of natural changes in women's physiology that increase the risk of insulin resistance, hyperlipidemia, and fat deposition in visceral organs due to inhibition of hepatic stellate cell proliferation and fibrogenesis owing to the antioxidant property of Estrogen, which reduces fatty acid oxidant and increases lipogenesis in the liver resulted into visceral fat deposition. Thus, the relationship -between premenopausal women and NAFLD development is strongly associated with hormonal changes[19].

5.2 Weight and BMI

Heish, *et al.,* in the general study of Japanese men, stated that compared to western people, Asian men have a higher inclination toward abdominal obesity. He also noted lower BMI and abdominal obesity are more dangerous factors[21]. Obesity is not the only fact that is related to NAFLD; lean individuals were also diagnosed with fatty liver in the present study; Sookoian et al. systematic review and meta-analysis supported the findings of our study as lean patients with normal BMI were diagnosed with NAFLD and shared a common altered metabolic and cardiovascular profile equally with obese with NAFLD[22]. Still, obesity is the significant reason for NAFLD, as fat distribution plays the main role in pathophysiological mechanisms in the case of metabolic disorders[23,24]. The retrospective population - based longitudinal cohort conducted by Vusirikala, *et al.,* in the United Kingdom stated that metabolically healthy individuals are more prone to developing NAFLD than normal-weight individuals[25]. Weight reduction is a helpful remedy for NAFLD with the support of lifestyle modification, moderate exercise, and safe therapies available.

As per classifications by the NIH and the World Health Organization (WHO) for BMI in White, Hispanic, and Black individuals, there is a certain classification as Normal BMI (the range of 18.5 to 24 kg/m^2), Overweight (25 to 29.9 kg/m^2), Obese [classified under class I (30 to 34.9 kg/m^2), and class II (35 to 39.9 kg/m^2), class III (greater than or equal to 40 kg/m^2)]. But for the Asian and South Asian populations, overweight is between 23 and 24.9 kg/m^2, and obesity is considered greater than 25 kg/m^2[26]. Considering this reference, in the present study, the NAFLD patients' mean score was 26.9982, 95% CI [26.022571, 27.973829] in Group A, whereas 26.8944 95% CI [25.813583, and 27.975217] in Group B. It indicates that most enrolled NAFLD patients were under the category of obesity. Sadroddin, *et al.*, supported the finding as a positive correlation between BMI, metabolic syndrome, and NAFLD cases in healthy blood donors in Kerman[27]. Another study by Pak N., *et al.,* reported a direct relationship between subcutaneous and visceral fat thickness measured by ultrasound and the occurrence of fatty liver with BMI[28]. BMI was statistically significant with the fatty liver; study findings suggested that higher BMI is an indicator and risk factor for the fatty liver; similar to Fan, *et al.*, supported the outcome[29]. After the accomplishment of intervention and 30 days of the follow-up period, the observed values as Group A - Mean score of 25.9912, 95% CI [25.146907, 26.835493] and Group B - Mean 25.5463, 95% CI [24.697087, 26.395513]. The study group patients (Group B) showed (P<0.001) remarkable results as compared to the control (Group A).

5.3 Lifestyle Modification

NAFLD is strongly linked with unhealthy lifestyles, primarily consuming extra calories, having an unhealthy diet, and lacking exercise. Alteration in diet and lifestyle modification like increased physical activity or moderate exercise is the first choice of treatment to lower the amount of fat and inflammation in the liver and enhance the metabolic profile, thereby lowering the risk of cardiac and liver disorders[30]. The present study provided accurate information and counseling to patients in both groups comprising overall behavior, diet regime, and moderate exercise[31]. In the control group, diet regime and moderate exercise were statistically significant (P>0.001). It means that lifestyle modification in diet and exercise is beneficial for NAFLD. A restricted calorie diet greatly affects a person's body physiology and behavior. Carneros D., *et al.,*[32] stated that a diet regime chronically reduces energy intake BY 15 – 40 % with a balanced diet with adequate vitamins and minerals. It plays a vital role in weight reduction and cutting hepatic fat, so a diet regime is considered the main factor in nutritional interventions for patients with NAFLD[32]. Moderate exercise stimulates a complex system of energy exchange between muscle and liver. This energy transfer increases amino acid metabolism, specifically branched-chain amino acids from muscle, and controls metabolic actions in the liver that induce lipolysis. Moderate exercise stimulates the release of signaling substances like myokines from muscle. It accelerates the release of other elements from the liver that control metabolic processes in the liver and the rest of the body[33,34].

6. Discussion of Physical, Haematological and Biochemical Parameter

6.1 SGOT (AST) and SGPT (ALT)

In the SGOT (AST) readings before the intervention, the mean value in all groups was almost the same, not having normal values, and the SD was identical except in the external control group. When comparing the range of values, it was found that the changes in the intervention group were significant as compared to other groups (95% CI - 49.35 - 46.62, P = 0.252). The percentage difference between the intervention vs. control group, i.e., ≥ 34%, and intervention vs. external control, i.e., ≥ 27%. The mean values of SGPT (ALT), except for the external control group, remaining groups had almost identical values before intervention. SD values were also substantially identical. After comparing the range of values, it was observed that intervention group changes were found significant as compared to other groups (95% CI - 50.92 - 46.54, P = 0.463). The percentage difference between the intervention vs. control group, i.e., ≥36%, and intervention vs. external control, i.e., ≥ 22%. Alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl-transpeptidase levels are indirect markers of non-alcoholic fatty liver disease and are elevated in this condition. The present study was consistent with findings reported by Papatheodoridis G. V., *et al*[3,35].

Many studies have shown that phlebotomy can lower liver enzymes. Hepatocellular necrosis appears to be reduced when iron levels are reduced with phlebotomy. Experiments have shown that lowering oxidative stress and necrosis lowers apoptosis and improves the health of liver cells. Oxidative stress can be reduced by lowering iron liver storage by phlebotomy. An improvement in insulin resistance can also decrease liver glucose synthesis, one mechanism of NAFLD steatosis development[36-38].

However, compared to the control and external control group, *Sira Vedha* (Phlebotomy) produced better results with the current methodology. The liver is formed by blood (S. Sha 4/25) and the liver (*Yakrut*) is *Mulasthana* of *Raktavaha Srotas* (S. Sha 9/12). *Shalyatantra* considers *Sira Vedh* to be half treatment (S. Sha 8/23). During bloodletting, the body expels impure blood first. Thus, in the current investigation, bloodletting with just 65 ml (1 Anjali) of blood was done at a 15-day interval, as is recommended for significant amounts of morbid *Dosha* (A.H.S. 27). Thus, *Sira Vedha* (Phlebotomy) reduces and relieves venous congestion, facilitates venous drainage, and improves oxygenated blood supply near the applied area, allowing the expulsion of morbid humor (vitiated *Doshas*, particularly vitiated *Pitta*, and other toxins) accumulated as a result of inflammatory reaction, thereby alleviating NAFLD symptoms.

7. Lipid Profile

The hypolipidaemic effect is one of the positive impacts of bloodletting. Total cholesterol and LDL levels appear significantly lower in regular blood donors. Regular blood donation appears to be linked to reducing serum lipids. In the present study, the mean value observed was 149.81 ± 10.71, 95% CI [146.89 - 152.73] in total cholesterol, 53 ± 4.08, 95% CI [52.44 - 54.66] HDL, in triglyceride 142.33 ± 35.78, 95% CI [132.56 - 152.1] after intervention in Group B, in totality 38%, 53% and 32% positive changes occurred. Uche E. I., *et al.,* quote that a significant reduction was induced in mean total cholesterol and low-density lipoprotein levels in persons with regular bloodletting. The regular bloodletting process may lower iron stores, which lowers lipid peroxidation because high iron levels play a main role in lipid oxidation and bloodletting reduces iron levels that, ultimately reducing oxidation of LDL[39-41].

Some other researchers, Van jaarsveld H., *et al.*,, Clement A. N. J., *et al.,* and Eshete E. A., *et al.,* have also supported this hypolipemic effect of bloodletting at regular intervals and regular bloodletting through blood donation helps to lower cholesterol and triglyceride levels[42-44].

According to Turankar S. A., *et al*., the significant reduction in total cholesterol, TG, LDL, and VLDL levels after seven days of bloodletting may be because of the utilization of cholesterol and TG to synthesize new cells in the process of erythropoiesis. Increased demand and less supply of cholesterol by the body cholesterol pool is another probable mechanism behind the lipid-lowering effect of bloodletting. This effect of bloodletting gets sustained even 30 days after the procedure[45]. The same observation is noted in the current study. Even after one month, the sustained effect of cholesterol on lipid profile shows that cholesterol is still used for erythropoiesis. The rate of cholesterol regeneration may not be sufficient to maintain appropriate lipid profile levels. A 53% increase in HDL cholesterol levels compared to baseline indicates that the peripheral transfer of cholesterol back to the liver and subsequently to the circulation, known as reverse cholesterol transport, is still occurring. Increased peripheral transport of HDL-cholesterol to fulfill the increased requirement of erythropoiesis could suggest an additional positive effect. The observation in the present study was in agreement with Kumar H., Mayer D. G., and Bhardwaj[46-48]. The erythropoiesis after bloodletting consumes iron and leads to a reduction in serum ferritin[49]. Iron is a significant catalyst for radical generation *in vitro*, according to Gutteridge JM and Halliwell B in “Iron hypothesis”[50]. The Fenton reaction, which is catalyzed by iron, produces a variety of powerful oxidants that can cause lipid oxidation[51,52].

As a result, more oxidative lipid products are produced, which leads to increased HDL transport to the liver, resulting in higher HDL levels. In this regard, the present study on the effect of *Sira Vedha* (Phlebotomy) at Right Elbow Joint in NAFLD is consistent with the previous studies conducted by Tabash A. M., *et al.,* Gebre-Yohannes A., *et al.,* Tuomainen T. P., *et al.,* Van Hoydonck P. G. A., *et al*[53-56].

8. Limitation of the Study

For comparison with the interventional group, secondary data (external control) was employed.

9. Conclusion

After comparing the baseline data with endpoint values in all the groups, physical, biochemical, and hematological parameters were statistically significant. In the Intervention group, there was a significant effect of Right Elbow Joint on SGOT, SGPT, Sr. Cholesterol, HDL, Sr. Triglyceride and BMI suggesting that *Siravedha* (Phlebotomy) at Right Elbow Joint was found to be more efficacious as compared to control group and external control.

10. Acknowledgements

We are thankful to Dr. S. S. Patel, Chief Coordinator, Datta Meghe Institute of Higher Education and Research, for the guidance. We also thankful to patients who gave consent to publish the data.

11. Statement of Ethics

An informed consents were obtained for this minimally invasive treatment modality. All the patients gave their informed consent for the publication of this original research.

12. Funding Sources

Datta Meghe Institute of Higher Education and Research (Deemed to be University), Wardha, Maharashtra, India – 442001.

13. Authors' Contribution Statement

All authors contributed for original article. Dr. Gaurav Sawarkar made the primary draft of the manuscript and the final manuscript. *Ayurveda* Treatment were advised by Dr. Punam Sawarkar and an assessment of the patient (before and after treatment) was also done by her. Dr. Priti Desai gave valuable inputs for corrections in the draft manuscript.

14. References

1. Organisation mondiale de la Santé. Manuel d'investigation des flambées épidémiques d'étiologie chimique présumée : orientation pour l'investigation et la régulation [Internet]. Genève: Organisation mondiale de la Santé; 2022 [cited 2022 May 12].
2. Khodadoostan M, Zamanidoost M, Shavakhi A, Sanei H, Shahbazi M, Ahmadian M. Effects of phlebotomy on liver enzymes and histology of patients with nonalcoholic fatty liver disease. Adv Biomed Res. 2017; 6:12. https://doi.org/10.4103/2277-9175.200787 PMid:28299304 PMCid:PMC5343605
3. Lainé F, Ruivard M, Loustaud-Ratti V, Bonnet F, Calès P, Bardou-Jacquet E, *et al.* Metabolic and hepatic effects of bloodletting in dysmetabolic iron overload syndrome: A randomized controlled study in 274 patients. Hepatol Baltim Md. 2017; 65(2):465–74. https://doi.org/10.1002/hep.28856 PMid:27685251
4. Rawal M, Chudasma KM, Vyas RV, Parmar BP. Effect of vasantic vaman and other panchakarma procedures on disorders of various systems. Ayu. 2010; 31(3):319–24. https://doi.org/10.4103/0974-8520.77160 PMid:22131733 PMCid:PMC3221065
5. Raval HN, Thakar AB. Role of raktamokshana by jalaukavacharana and siravedhana in the management of vicharchika (eczema). Ayu. 2012; 33(1):68–72. https://doi.org/10.4103/0974-8520.100314 PMid:23049187 PMCid:PMC3456867
6. Raut SY, Rasale PL, Tambade AB. A study of changes in RBC indices after raktamokshana w.s.r. Siravedha. Int J Ayurvedic Med. 2013; 4(3). https://doi.org/10.47552/ijam.v4i3.263
7. Vaneet Kumar J, Dudhamal TS, Gupta SK, Mahanta V. A comparative clinical study of siravedha and agnikarma in management of gridhrasi (sciatica). Ayu. 2014; 35(3):270–6. https://doi.org/10.4103/0974-8520.153743 PMid:26664236 PMCid:PMC4649569
8. Rana J. Clinical evaluation of siravedha in the management of gridhrasi w.s.r to sciatica. World J Pharm Res. 2017; 1181–92. https://doi.org/10.20959/wjpr20177-8795
9. Sutrasthan AH. Chapter 27: Siravyadha Vidhi Venesetion (Blood Letting) 27/44, 316:352.
10. Beaton MD, Chakrabarti S, Levstik M, Speechley M, Marotta P, Adams P. Phase II clinical trial of phlebotomy for non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2013; 37(7):720–9. https://doi.org/10.1111/apt.12255 PMid:23441892

11. Burger HU, Gerlinger C, Harbron C, Koch A, Posch M, Rochon J, *et al*. The use of external controls: To what extent can it currently be recommended? Pharm Stat. 2021; 20(6):1002–16. https://doi.org/10.1002/pst.2120 PMid:33908160
12. Abenavoli L, Milic N, Peta V, Alfieri F, De Lorenzo A, Bellentani S. Alimentary regimen in non-alcoholic fatty liver disease: Mediterranean diet. World J Gastroenterol WJG. 2014; 20(45):16831–40. https://doi.org/10.3748/wjg.v20.i45.16831 PMid:25492997 PMCid:PMC4258553
13. Fan JG, Cao HX. Role of diet and nutritional management in non-alcoholic fatty liver disease. J Gastroenterol Hepatol. 2013; 28(S4):81–7. https://doi.org/10.1111/jgh.12244 PMid:24251710
14. Marchesini G, Petta S, Dalle Grave R. Diet, weight loss, and liver health in nonalcoholic fatty liver disease: Pathophysiology, evidence, and practice. Hepatology. 2016; 63(6):2032–43. https://doi.org/10.1002/hep.28392 PMid:26663351
15. Fernández T, Viñuela M, Vidal C, Barrera F. Lifestyle changes in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis. Strnad P, editor. PLOS ONE. 2022; 17(2):e0263931. https://doi.org/10.1371/journal.pone.0263931 PMid:35176096 PMCid:PMC8853532
16. Mahaling DU, Basavaraj MM, Bika AJ. Comparison of lipid profile in different grades of non-alcoholic fatty liver disease diagnosed on ultrasound. Asian Pac J Trop Biomed. 2013; 3(11):907–12. https://doi.org/10.1016/S2221-1691(13)60177-X
17. Khanal U, Paudel B, Gurung G, Hu YS, Kuo CW. Correlational study of nonalcoholic fatty liver disease diagnosed by ultrasonography with lipid profile and body mass index in adult nepalese population. J Med Ultrasound. 2019; 27(1):19. https://doi.org/10.4103/JMU.JMU_53_18 PMid:31031531 PMCid:PMC6445041
18. Ariya M, Koohpayeh F, Ghaemi A, Osati S, Davoodi SH, Razzaz JM, *et al*. Assessment of the association between body composition and risk of non-alcoholic fatty liver. PLoS ONE. 2021; 16(4):e0249223. https://doi.org/10.1371/journal.pone.0249223 PMid:33793621 PMCid:PMC8016222
19. Summart U, Thinkhamrop B, Chamadol N, Khuntikeo N, Songthamwat M, Kim CS. Gender differences in the prevalence of nonalcoholic fatty liver disease in the Northeast of Thailand: A population-based cross-sectional study. F1000 Research. 2017; 6:1630. https://doi.org/10.12688/f1000research.12417.2 PMid:29093809 PMCid:PMC5645706
20. Ahuja M. Age of menopause and determinants of menopause age: A PAN India survey by IMS. J -Life Health. 2016; 7(3):126. https://doi.org/10.4103/0976-7800.191012 PMid:27721640 PMCid:PMC5051232
21. Hsieh SD, Yoshinaga H, Muto T, Sakurai Y, Kosaka K. Health risks among Japanese men with moderate body mass index. Int J Obes Relat Metab Disord J Int Assoc Study Obes. 2000; 24(3):358–62. https://doi.org/10.1038/sj.ijo.0801157 PMid:10757631
22. Sookoian S, Pirola CJ. Systematic review with meta-analysis: Risk factors for non-alcoholic fatty liver disease suggest a shared altered metabolic and cardiovascular profile between lean and obese patients. Aliment Pharmacol Ther. 2017; 46(2):85–95. https://doi.org/10.1111/apt.14112 PMid:28464369
23. Godoy-Matos AF, Silva Júnior WS, Valerio CM. NAFLD as a continuum: From obesity to metabolic syndrome and diabetes. Diabetol Metab Syndr. 2020; 12(1):60. https://doi.org/10.1186/s13098-020-00570-y PMid:32684985 PMCid:PMC7359287
24. Cho JY, Chung TH, Lim KM, Park HJ, Jang JM. The impact of weight changes on nonalcoholic fatty liver disease in adult men with normal weight. Korean J Fam Med. 2014; 35(5):243–50. https://doi.org/10.4082/kjfm.2014.35.5.243 PMid:25309705 PMCid:PMC4192788
25. Vusirikala A, Thomas T, Bhala N, Tahrani AA, Thomas GN, Nirantharakumar K. Impact of obesity and metabolic health status in the development of non-alcoholic fatty liver disease (NAFLD): A United Kingdom population-based cohort study using the health improvement network (THIN). BMC Endocr Disord. 2020; 20(1):96. https://doi.org/10.1186/s12902-020-00582-9 PMid:32605642 PMCid:PMC7325099
26. Weir CB, Jan A. BMI Classification Percentile And Cut Off Points. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.
27. Lahsaee S, Ghazizade A, Yazdanpanah M, Enhesari A, Malekzadeh R. Assessment of NAFLD cases and its correlation to BMI and metabolic syndrome in healthy blood donors in Kerman. Gastroenterol Hepatol Bed Bench. 2012; 5(4):183–9.
28. Pak N, Allahverdi B, Moslemi S, Rostami P, Sayarifard F, Mehdizadeh M, *et al*. Relationship Between Body Mass Index, Fatty Liver, Lipids Profile, Carotid Intima-Media Thickness and Subcutaneous and Visceral Fat Determined by Ultrasound. Iran J Pediatr [Internet]. 2020; 30(1). https://doi.org/10.5812/ijp.96605

29. Fan R, Wang J, Du J. Association between body mass index and fatty liver risk: A dose-response analysis. Sci Rep. 2018; 8(1):15273. https://doi.org/10.1038/s41598-018-33419-6 PMid:30323178 PMCid:PMC6189125
30. Francque SM, Marchesini G, Kautz A, Walmsley M, Dorner R, Lazarus JV, *et al*. Non-alcoholic fatty liver disease: A patient guideline. JHEP Rep. 2021; 3(5):100322. https://doi.org/10.1016/j.jhepr.2021.100322 PMid:34693236 PMCid:PMC8514420
31. Shin SW, Jung SJ, Jung ES, Hwang JH, Kim WR, So BO, *et al*. Effects of a lifestyle-modification program on blood-glucose regulation and health promotion in diabetic patients: a randomized controlled trial. J Lifestyle Med. 2020; 10(2):77–91. https://doi.org/10.15280/jlm.2020.10.2.77 PMid:32995335 PMCid:PMC7502894
32. Carneros D, López-Lluch G, Bustos M. Physiopathology of lifestyle interventions in non-alcoholic fatty liver disease (NAFLD). Nutrients. 2020; 12(11):3472. https://doi.org/10.3390/nu12113472 PMid:33198247 PMCid: PMC7697937
33. Ennequin G, Sirvent P, Whitham M. Role of exercise-induced hepatokines in metabolic disorders. Am J Physiol Endocrinol Metab. 2019; 317(1):E11–24. https://doi.org/10.1152/ajpendo.00433.2018 PMid:30964704
34. Severinsen MCK, Pedersen BK. Muscle–organ crosstalk: The emerging roles of myokines. Endocr Rev. 2020; 41(4):594–609. https://doi.org/10.1210/endrev/bnaa016 PMid:32393961 PMCid:PMC7288608
35. Papatheodoridis GV, Goulis J, Christodoulou D, Manolakopoulos S, Raptopoulou M, Andrioti E, *et al*. High prevalence of elevated liver enzymes in blood donors: Associations with male gender and central adiposity. Eur J Gastroenterol Hepatol. 2007; 19(4):281–7. https://doi.org/10.1097/MEG.0b013e328011438b PMid:17353691
36. Facchini FS, Hua NW, Stoohs RA. Effect of iron depletion in carbohydrate-intolerant patients with clinical evidence of nonalcoholic fatty liver disease. Gastroenterology. 2002; 122(4):931–9. https://doi.org/10.1053/gast.2002.32403 PMid:11910345
37. Sumida Y, Kanemasa K, Fukumoto K, Yoshida N, Sakai K, Nakashima T, *et al*. Effect of iron reduction by phlebotomy in Japanese patients with nonalcoholic steatohepatitis: A pilot study. Hepatol Res Off J Jpn Soc Hepatol. 2006; 36(4):315–21. https://doi.org/10.1016/j.hepres.2006.08.003 PMid:16971174
38. Dongiovanni P, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. J Hepatol. 2011; 55(4):920–32. https://doi.org/10.1016/j.jhep.2011.05.008 PMid:21718726
39. Uche E, Adediran A, Damulak O, Adeyemo T, Akinbami A, Akanmu A. Lipid profile of regular blood donors. J Blood Med. 2013; 4:39–42. https://doi.org/10.2147/JBM.S42211 PMid:23717053 PMCid:PMC3663474
40. Bharadwaj RS. A Study of Lipid Profiles Among Male Voluntary Blood Donors in Chennai City. 2005; 30(1):2.
41. Meyers DG, Strickland D, Maloley PA, Seburg JK, Wilson JE, McManus BF. Possible association of a reduction in cardiovascular events with blood donation. Heart. 1997; 78(2):188–93. https://doi.org/10.1136/hrt.78.2.188 PMid:9326996 PMCid:PMC484902
42. van Jaarsveld H, Pool GF. Beneficial effects of blood donation on high density lipoprotein concentration and the oxidative potential of low density lipoprotein. Atherosclerosis. 2002; 161(2):395–402. https://doi.org/10.1016/S0021-9150(01)00638-4 PMid:11888523
43. Clement ANJ, Vincent VS, Shey ND, Afoni BE, Marcelin NN. A cross-sectional study on the evaluation of the lipid profile of regular blood donors in the buea regional hospital, Cameroon. Cardiol Cardiovasc Res. 2017; 1(3):76.
44. Eshete EA, Weldemariam TZ. Hematological and lipid profiles of blood donors at red cross center in Addis Ababa. Ethiop Med J. 2016; 54(1):21–5.
45. Turankar SA, Turankar AV, Vaidya S, Balpande S. Effect of blood donation on lipid profile in healthy volunteers:A short-term pilot study. J Evol Med Dent Sci. 2018; 7(33):3670–4. https://doi.org/10.14260/jemds/2018/824
46. Kumar H. Repeated blood donation effective in treating hyperlipidemia. J Assoc Physicians India. 1994; 42(6):468–9.
47. Meyers DG, Strickland D, Maloley PA, Seburg JK, Wilson JE, McManus BF. Possible association of a reduction in cardiovascular events with blood donation. Heart Br Card Soc. 1997; 78(2):188–93. https://doi.org/10.1136/hrt.78.2.188 PMid:9326996 PMCid:PMC484902
48. Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem. 1973; 19(12):1350–6. https://doi.org/10.1093/clinchem/19.12.1350 PMid:4757363
49. Frei B. Reactive oxygen species and antioxidant vitamins: Mechanisms of action. Am J Med. 1994; 97(3A):5S-13S; discussion 22S-28S. https://doi.org/10.1016/0002-9343(94)90292-5 PMid:8085584
50. Salonen JT, Korpela H, Nyyssönen K, Porkkala E, Tuomainen TP, Belcher JD, *et al*. Lowering of body iron stores by blood letting and oxidation resistance of serum lipoproteins: a randomized cross-over trial in male smokers. J Intern Med. 1995; 237(2):161–8. https://doi.org/10.1111/j.1365-2796.1995.tb01156.x PMid:7852918

51. McCord JM. Is iron sufficiency a risk factor in ischemic heart disease? Circulation. 1991 ; 83(3):1112–4. https://doi.org/10.1161/01.CIR.83.3.1112 PMid:1999020
52. Sullivan JL. Blood donation may be good for the donor. Iron, heart disease, and donor recruitment. Vox Sang. 1991; 61(3):161–4. https://doi.org/10.1111/j.1423-0410.1991.tb00940.x PMid:1807057
53. M. Tabash A, M. Afana W, M. Elregeb A, Abu Eid S, M. AbuMustafa A. Assessment of Lipid and Hematological Profile among Blood Donors in European Gaza Hospital, Palestine. Cardiol Cardiovasc Med [Internet]. 2019; 03(04). https://doi.org/10.26502/fccm.92920063
54. Gebre-Yohannes A, Rahlenbeck SI. Coronary heart disease risk factors among blood donors in northwest Ethiopia. East Afr Med J. 1998; 75(9):495–500.
55. Tuomainen TP, Salonen R, Nyyssönen K, Salonen JT. Cohort study of relation between donating blood and risk of myocardial infarction in 2682 men in eastern Finland. BMJ. 1997; 314(7083):793–4. https://doi.org/10.1136/bmj.314.7083.793 PMid:9080998 PMCid:PMC2126176
56. Van Hoydonck PGA, Schouten EG, Hoppenbrouwers KPM, Temme EHM. Is blood donation induced low iron status associated with favourable levels of OxLDL, s-ICAM-1, sVCAM-1 and vWF-antigen in healthy men. Atherosclerosis. 2004; 172(2):321–7. https://doi.org/10.1016/j.atherosclerosis.2003.11.006 PMid:15019542