Original Article

A Double Blind, Randomized, Placebo Controlled Clinical Study To Evaluate The Efficacy And Safety Of Tfn34 Tablets For Individuals With Subclinical And Clinical Hypothyroidism

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Abstract

Hypothyroidism is one of the commonly presented medical complications addressed.There are Clinical symptoms that happens to differ from mild unspecific symptoms namely cold intolerance, tiredness, obstipation to life-threatening signs like myxedema&lack of vitality.The current study involved on designing as well as evaluating the clinical efficacy of the aforementioned prescription on poly-herbal formulation of oral medicationfor effective management of hypothyroidism. This study is planned to evaluate the efficacy and safety of TFN34 tablets for Individuals with subclinical & clinical hypothyroidism as assessed by clinical and laboratory methods including Zulewski Index questionnaire. Evaluating the treatment regime effect on hypothyroidism management and treatment that monitoring the level of FT3, FT4 and TSH is the secondary objective for identifying the compliance &AE to the study.The treatment regime comprised of administering TFN34 tablets which was developed with polyherbal formulation developed and manufactured by Apex Laboratories Private Limited, Chennai and the clinical trial was conducted at SRM Medical College Hospital & Research Centre,Kattankulathur.The clinical trial was intended to determine the efficacy &the safety of administering TFN34 tablets in the treatment and management of hypothyroidism cases among 60 patients by conducting a placebo controlled, randomized double blind study.

Keywords: Hypothyroidism, placebo, TFN34, randomization, subclinical and clinical hypothyroidism etc.

1. Introduction

Among major endocrine disorders, hypothyroidism is one of the commonly presented medical complications addressed.There are Clinical symptoms that happens to differ from mild unspecific symptoms namely cold intolerance, tiredness, obstipation to life-threatening signs like myxedema&lack of vitality.With regards to myxedema symptoms, there lies greater sensitivity for pharmacotherapy, followed with symptoms notably being

confusion, areflexia, risk that are associated to death &megacolon(Chung GyoSeo et al., 2019). Thyrotropin is a primary hypothyroidism pituitary derived thyroid stimulating hormone (TSH) that has increased over 10 mIU/L in free thyroxin (T4) with a simultaneous decrease(Calissendorff&Falhammar, 2020). It is observed that most of the patients have quantifiable auto-antibodies against TPO ab (thyroid peroxidase) and important enzymes in the synthesis of thyroidhormonesthat considered as a marker for autoimmune thyroid disease. With regards to Primary hypothyroidism which might be clinical, wherein free T_4 (FT_4) represented with reduced levels of TSH is increased subclinical in which the FT_4 levels are normal whereas there is an elevated level in the TSH ranges. FT_4 is decreased in secondary hypothyroidism and TSH has been normal or it remains lower. Due to chronic autoimmune thyroiditis, primary hypothyroidism is caused commonly that could be effectively managed with radioiodine treatment &thyroidectomy (Manolis et al., 2020).

The overall incidence in the clinical hypothyroidism is reported to be between 0.5-1.9% among women and only <1% among the males & in respect to the subclinical cases was found to be between 3-13.6% among females &0.7-5.7% among the males. There is a greater deal of differentiating clinical from that of subclinical hypothyroidism cases, wherein the underlying clinical symptoms might be life threatening & might lead to uneventful occurrences like comatose stage. However, when considering similar situation for subclinical symptoms seems to be less or even be absent in predominant cases. There is a greater likelihood for patients who are under subclinical hypothyroidism to transform into clinical and from the recent literature reports indicated with patients with clinical presentations such as hyperlipidemiafollowed with a much greater risks associated with patients' susceptible to CVD, even heart failures, neuromuscular &somatic symptoms, reproductive and other systemic complications.

Bahunia purpurea (Panda & Kar, 1999), Guggulu (Commiphoramukul) (Panda & Kar, 2005; Tripathi et al., 1984), varuna (kaur et al., 2017) Ashwagandha (Withaniasomnifera) (Panda & Kar, 1998) (Tarun Sharma et al., 2015), Kanchanar (Bahunia variegate) (Tarun Sharma et al., 2015; Veena et al., 1975),Dasamool (Panja et al., 2003), twak (Cinnamomum zeylanicum Nees)(Bhist& Dwivedi, 2017)(Samir et al., 2009) (Ranasinghe et al., 2013), triphala- Fr (5)(6), trikatu- Fr. &Rz, Khadira (Acacia catechu) (Sidney et al., 2015), Twakpatra (Cinnamomum tamala) (Devi et al., 2007) (Peepre et al., 2014), Satavari (Asparagus racemosus) (KONGKANERAMIT et al., 2011)(Prakash et al., 2015), Brahmi (Bacopa monnieri) (Tarun Sharma et al., 2015) like herbs and also other notable herbal compositions like Apamarga (Achyranthes aspera) Tahiliani& Kar (2000), Kustha (Saussurealapa) (Chaturvedi et al., 1993) (Apurva Patel, 2009) are used in Ayurvedic medicine in many centuries. There was a rise in the concentration of serum T4 that showed by Ashwagandha (Withaniasomnifera) when both the concentration of serum T3 and T4 was a rise that enhanced by Kanchanar (Bhahunia purpurea) in a study by Panda and Kar (1999).The activity of hepatic glucose 6 phosphatase enzyme was increased by both the plant and they had certain antiperoxidative effects. T4 concentration can be increased by Brahmi (Bacopa monnieri) like herbs (Kar et al., 2002) that suggesting its thyroid stimulating activity in Tripathi et al's (1984) research which suggested that the T4 conversion can be increased to T3 by Guggulu through the iodine uptake into thyroxin&proteolytic activity. Potentially, hypothyroidism can be ameliorated by Guggulu and they have potent anti-oxidative properties (Panda & Kar, 2005).

Furthermore the application of oral administration of medicines in Ayurvedic treatment has gained immense level of interest for ameliorating hypothyroidism condition with minimal to no side-effects. The current study involved on designing as well as evaluating the clinical efficacy of the aforementioned prescription on poly-herbal formulation of oral medicationforeffective management of hypothyroidism.

This was achieved by conducting a double-blind placebo controlled clinical study consisting of 60 subjects who fulfil the subject selection criteria. According to the study, all subjects had been advised with TFN34 tablets'administration or with placebo that identically looking at a dosage of 2 tablets twice in a daily for six months. The primary study objective involved with assessment of effects on Ayurvedic medication (TFN34) on rates of recovery from hypothyroidism.This study is planned to evaluate the efficacy and safety of TFN34 tablets for Individuals with subclinical & clinical hypothyroidism as assessed by clinical and laboratory methods

including Zulewski Index questionnaire. Evaluating the treatment regime effect on hypothyroidism management and treatment that monitoring the level of FT3, FT4 and TSH is the secondary objective for identifying the compliance &AE to the study.

Methodology

The treatment regime comprised of administering TFN34 tablets which was developed with polyherbal formulation developed and manufactured by Apex Laboratories Private Limited, Chennai andthe clinical trial was conducted at SRM Medical College Hospital & Research Centre,Kattankulathur. The clinical trial was intended to determine the efficacy & the safety of administering TFN34 tablets in the treatment and management of hypothyroidism casesamong 60 patientsby conducting a placebo controlled, randomized double blind study.

IEC (the Institutional Ethical Committee)had been carried out by the study as per the IEC approving protocol (Ref. No. NIMSUR/IEC/2020/036). For hypothyroidism, the result showed that every patient had been positive and they had been registered with their formal consents. In Figure 1, a detailed study plan has been rendered.

60 patients recruited and studied

Randomization

Placebo Arm

Dummy medicines with identical looking placebo at a dose of 2 tablets twice daily for 6 months.

Treatment Arm

Oral Administration of TFN34 Tablets

TFN34 tablet

Herbs	Part used	Quantity
Kanchanara(*Bauhinia variegata*)	Stem bark	150.00 mg
Guggul (*Commiphora mukul*)	Exudate	100.00 mg
Varuna (Crateva nurvula) 50 mg	Stem bark	50.00 mg
Triphala - *Fr*	Ayurvedic Shastric Medicine	50.00 mg
Trikatu – *Fr.* & *Rz*	Ayurvedic Shastric Medicine	50.00 mg
Aswagandha(*Withania somnifera*)	Root	40.00 mg
Brahmi (*Bacopa monnieri*)	Whole plant	30.00 mg
Tvak *(Cinnamomum zeylanicum)*	Bark	25.00 mg
Dasamool (Ten roots)	Roots	25.00 mg
Khadira *(Acacia catechu)*	Heart wood	20.00 mg
Satavari (*Asparagus racemosus*)	Tuberous root	20.00 mg
Twak patra (*Cinnamomum tamala*)	Leaves	15.00 mg

Inclusion Criteria

- Male and female subjects with aged between 18 to 60 years.
- Subjects with TSH Value ≥ 4.5
- Individual presenting with at least four of the symptoms like Fatigue, cold intolerance, Constipation, Dry rough & pale skin, Weight gain, Puffiness of face, Hoarseness, Muscle weakness, Muscle cramps and frequent muscle aches, Abnormal menstrual cycle, Dry, coarse thinning of hair, Hair loss, Slowed heart rate, Depression, Irritability, Impaired memory, Decreased libido. Subjects willing to sign informed consent and follow the study procedure.
- Has not participated in a similar investigation in the past four weeks.

Exclusion Criteria

- Subjects currently on levothyroxine, anti-thyroid drugs, amiodarone or lithium.
- Subjects with any clinically significant and uncontrolled systemic illnesses (including Gastrointestinal, Cardiac, Neurological, Respiratory, Immunological or endocrinological disorder) as judged by the investigator, which can hamper the participation of subjects in clinical study.
- Hospitalization for major illnesses/ elective surgery within 4 weeks
- Clinical diagnosis of any psychiatric or psychological disorders rendering the subject not to follow the study procedures.
- Pregnant or lactating women or women of child bearing potential not willing to use the established measures of contraception.

Follow-up

Baseline — 2nd month — 4th month — 6th month

Clinical assessment
Laboratory Investigation involving Haematological investigations (conducted at the end of each follow-up visit) **Biochemical investigations:** At the end of end of each follow-up visit)
Study specific Examinations (evaluated at entry and at the end of the study (6th month)- FT3 level; FT4 level, TSH, T3 level & T4 level

Case detection and subject selection

Volunteers who were participating were assessed for details that meets with inclusion – exclusion criteria. All subjects were routinely screened and subjected with prior clinical examination, Hematological and biochemical investigations. Before entering into the study, the subjects were prescreened by the investigator for the criteria indicated in the Subject Selection section. Only subjects who have qualified the screening and willing to participate in the study will be called for the study, and informed consent process is carried on. Patients' health history data was collected by competent authority of the trial site. All patients, who were admitted in this facility were identified for the symptomology of chronicity and any history of past disease and its treatment. Study proforma was routinely checked by the coordinator, to fill the missing data of the patient history and addressed queries. General and systemic physical examinations were done as a focused approach. Vitals were checked and recorded in the medical case files. The laboratory data was collected from the hospital EMR and patient case files. The inclusion and exclusion screening criteria used for this study are in Figure 1. Patients fulfilling all these criteria were made aware of the study and informed consent was taken from those who were willing to participate. Subsequently, the patients were randomized into the treatment and placebo arms.

Randomization

The patients were randomized into the treatment and the placebo groups in 1:1 ratio. The randomization was done with the help of computer-generated random number tables. Patients assigned with odd numbers were given placebo (Placebo Arm) and those assigned with even numbers received TFN34 (Treatment Arm).

Baseline investigation

Various hematological parameters like Hemoglobin, Total WBC count,
Differential Leucocytes count, Platelet count, Erythrocyte Sedimentation Rate, Absolute Eosinophil Count were monitored at the end of every month. Similarly **Biochemical investigations such as** Renal Function Test (RFT)

Serum creatinine (mg/dL) Blood Urea Nitrogen (BUN) (mg/dL) Urea (mg/dL),

Uric acid (mg/dL), Glomerular filtration rate (GFR), Liver Function Test (LFT), SGPT (U/L) SGOT (U/L) , Total Bilirubin (mg/dL), Direct Bilirubin (mg/dL), Indirect Bilirubin (mg/dL) ALP (IU/L), Albumin (g/liter), Blood Sugar Test: RBS (million cells/c mm) were also evaluated for the experimental and the plaebo arm. All the patients enrolled in the trial were evaluated clinically for the symptoms were done on Fatigue, cold intolerance, constipation, dry rough & pale skin, weight gain, puffiness of face, hoarseness, muscle weakness, muscle cramps and frequent muscle aches, abnormal menstrual cycle, dry, coarse thinning of hair, hair loss, slowed heart rate, depression, irritability, decreased libido, nervousness, excessive sleep, feeling of indigestion, memory lapse or foggy thinking and anxiety. Assessment of the parameters will be done on the scale of 0 - 3. (0-nil, 1-mild, 2-moderate, 3- severe).

Study Specific Laboratory investigation:

- FT3 level
- FT4 level
- TSH
- T3 level
- T4 level

Questionnaire to assess sub clinical and clinical hypothyroidism - Zulewski Index Score:

At Baseline, end of 2nd month, end of 4th month and end of 6th month.

Treatment and drug administration

Ayurvedic treatment comprised of oral administrations of TFN34 to the experimental subjects and were directed to take 2 tablets of TFN34 tablets or Placebo twice daily before food for a period of 6 months. Allocations were concealed using sealed envelopes. Outcome assessor is not blinded as only investigators and subjects were blinded in a double blind study. Apart from the above-mentioned treatment, no other intervention was provided to the patients.

Follow up monitoring and investigations

Some of the parameters were investigated on day 1 of administering the medicines. These included the serum levels of interleukin-6 (IL-6), C-reactive protein (hs-CRP) and tumor necrosis factor-alpha (TNF-α). On day 3, RT-PCR was repeated to assess the virological clearance. Patients who turned out negative for day 3 RT-PCR were excluded from further assessment of virological clearance through RT-PCR. Such patients were labelled as RT-PCR negative in the study proforma. For rest of the patients, nasopharyngeal swabs were again subjected to RT-PCR analysis for monitoring the virological clearance on day 7. In order to evaluate the effect of the treatment on the inflammatory response, serum levels of IL-6, TNF-α and hs-CRP and ESR were also assessed on day 7. Blood biochemical parameters, like, total leukocyte and differential leucocyte counts and liver and kidney function tests (AST, ALT, serum albumin, serum creatinine, total bilirubin, direct bilirubin) were repeated on day 7. Blood pressure, pulse rate, respiratory rate and oxygen saturation were monitored daily during the treatment. However, for study purpose, day 3 and day 7 vitals were recorded in the proforma.

Data collection

Data was collected at National Institute of Medical Sciences, Jaipur, Rajasthan, India. A proforma was made to collect the data. Data was cross-checked to ensure error proofing.

Investigations

Primary outcomes

Patient with clinical improvement in the symptoms Fatigue, cold intolerance, Puffiness of face, Hoarseness, Muscle weakness, Hair loss, Slowed heart rate, Depression, Irritability, Impaired memory and the points from the questionnaire recorded from the patients.

In addition, Blood levels of FT3 levels, FT4 levels, TSH, T3 levels, T4 levels were observed during the baseline investigation and the end of the 6 month follow-up period.

Secondary Endpoints:

- Compliance of the subject to the drug treatment and the incidence of events.

Statistical analysis

Data was analyzed through SPSS ver 23 (released 2015 IBM SPSS Statistics for windows, version 23.0. Armonk, NY: IBM Corp). All continuous variables were checked for the normality by the shapiro wilk test. Parametric continuous variables were presented by mean and SD. Non parametric continuous variables were depicted as a median. The nominal variables were analyzed by chi-square test.

Results

During mm-2022 to mm-2022, 70 patients were screened and enrolled for the study. Out of these 70 patients, 10 were not participating in the follow-up nor withdrew the consents. Therefore, the observations reported in the study comprised a total of 60 patients

Patients' Demographics

All the patient demographics are summarized for both experimental (TFN34) group and placebo group in Table 1. From the findings it could be inferred that the following experimental study was conducted only in female. Majority of the respondents showed signs of healthier lifestyle with very minimal level of participants were reported.

Table1: patient Demographics

Demographic profile					
RANDOMISATION		Standard		TFN	
		Frequency	Percent	Frequency	Percent
SEX	Female	30	100.0	29	96.7
	Male	-	-	1	3.3
	Total	30	100.0	30	100.0
SMOKING	NO	30	100.0	29	96.7
	YES	-	-	1	3.3
	Total	30	100.0	30	100.0
DRINKING	NO	30	100.0	29	96.7
	YES	-	-	1	3.3
	Total	30	100.0	30	100.0
DIET	KETO DIET	1	3.3	22	73.3
	MIXED DIET	29	96.7	8	26.7
	Total	30	100.0	30	100.0
ALLERGIES	NIL	25	83.3	28	93.3
	PEANUT ALLERGY	1	3.3	-	-
	PENICILLIN	1	3.3	1	3.3
	SEA FOOD	2	6.7	1	3.3
	UNKNOWN	1	3.3	-	-
	Total	30	100.0	30	100.0

Hematological findings between both the groups were investigated and was compared during their baseline investigation and after the 6 months followup period.

Table 2. Hematological investigations

RANDOMISATION		Mean	Std. Deviation	Sig. value	Mean	Std. Deviation	Sig. value
		Standard			TFN		
Pair 1	HAEMOGLOBIN-ON ENTRY	11.8333	1.05089	.148	11.6233	1.18545	.448
	HAEMOGLOBIN-6TH MON	11.6200	.91742		11.6567	1.05133	
Pair 2	TOTAL WBC-ON ENTRY	9176.6667	1147.01512	.410	9170.0000	1856.98124	.303
	TOTAL WBC-6TH MON	9250.0000	1351.56359		9420.0000	1495.83329	
Pair 3	LYMPHOCYTES-ON ENTRY	29.7000	6.51867	.307	28.3667	6.07132	.367
	LYMPHOCYTES-6TH MONTH	28.8000	7.19387		28.9667	6.84046	
Pair 4	NEUTROPHIL-ON ENTRY	57.7000	14.65735	.373	57.7667	13.32792	.202
	NEUTROPHIL-6TH MONTH	56.7333	14.45071		55.1333	13.45166	
Pair 5	MONOCYTES-ON ENRTY	4.4000	2.31338	.383	3.9667	2.07586	.280
	MONOCYTES-6 TH MONTH	4.2000	2.41261		4.3333	2.30940	
Pair 6	EOSINOPHIL-ON ENRTY	1.4000	1.19193	.018	1.4667	1.13664	.153
	EOSINOPHIL-6TH MONTH	2.1333	1.40770		1.8000	1.24291	
Pair 7	ESR-ON ENTRY	9.4667	4.45462	.052	10.4000	5.41135	<.001
	ESR-6TH MON	7.7333	3.20488		6.1000	2.99828	

In this double randomized design from pair-wise distribution test showcased that the renal function test among the patients observed that though the serum creatinine levels were within the normal reference range, it was higher and serum creatinine & eGFR was lowered significantly in the TFN group when compared to the placebo group.

Baseline Biochemical investigations:Renal Function Test (RFT)

RANDOMISATION		Mean	Std. Deviation	Sig. value	Mean	Std. Deviation	Sig. value
		Standard			TFN		
Pair 1	SR CREATININE-BASELINE	.7890	.09632	.305	.8017	.11201	.236
	SR CREATININE-FOLLOW	.7770	.09952		.7807	.08225	
Pair 2	BUN(6-24 mg/dl)-BASELINE	15.1333	4.51613	.297	15.1667	3.50451	.301
	BUN(6-24 mg/dl)-FOLLOW	14.5667	4.43095		14.8000	4.04628	
Pair 3	SR UREA(15 - 40)-BASELINE	29.9000	7.11167	.337	30.5333	4.95311	.123
	SR UREA(15 - 40)-FOLLOWUP	28.8333	10.22196		27.7667	10.62750	
Pair 4	URIC ACID-BASELINE	3.7233	.82114	.436	3.6867	.96944	.075
	URIC ACID-FOLLOW	3.7567	.83859		3.3800	.98240	
Pair 5	GFR-BASELINE	109.3333	10.56453	.072	111.3333	11.66585	.474
	GFR-FOLLOW	113.3333	9.94236		111.5000	10.91898	

From Table 4. The biochemical investigation of the Liver function test was assessed for both TFN group and the placebo. It was observed that the Mean of total bilirubin levels was decreased considerably in TFN group when compared with the placebo with p value found to be extremely significantly. Mean value for SGOT, SGPT elevated in the followup period when compared to the baseline investigation.

Biochemical investigations:Liver Function Test (LFT)

RANDOMISATION		Mean	Std. Deviation	Sig. value	Mean	Std. Deviation	Sig. value
		Standard			TFN		
Pair 1	SGPT(5-35)-BASELINE	27.6000	5.67876	.250	29.6333	3.05674	.361
	SGPT(5-35)-FOLLOWUP	28.1667	4.69103		29.3667	3.16754	
Pair 2	SGOT (8-40)-BASELINE	22.8500	7.56511	.068	22.9933	6.90672	.229
	SGOT (8-40)-FOLLOW UP	25.3133	6.17016		24.1000	5.93958	
Pair 3	TOTAL BILIRUBIN(mg/dl)-BASELINE	.8100	.14704	.315	.8000	.17221	.180
	TOTAL BILIRUBIN(mg/dl)-FOLLOW	.8300	.15570		.7500	.19608	
Pair 4	DIRECT BILIRUBIN(mg/dl)-BASELINE	.4167	.16206	.126	.4000	.17221	.206

	DIRECT BILIRUBIN(mg/dl)-FOLLOW	.3667	.16884		.4400	.16733	
Pair 5	ALP (IU/L)-BASELINE	112.2667	44.81682	.109	131.2333	31.41237	.011
	ALP (IU/L)-FOLLOW	98.2333	41.01361		98.5000	58.02184	
Pair 6	ALBUMIN (g/ Liter)-BASELINE	3.9300	.56026	.423	4.1033	.62614	.466
	ALBUMIN (g/ Liter)-FOLLOW	3.9567	.63771		4.0900	.65724	

In regards to the blood sugar levels observed from the RBS-baseline versus the followup, it was observed that the mean RBS was significantly lowered in the followup period in TFN group, contrarily the RBS elevated in the followup period of the study for placebo group.

Biochemical investigations:Blood Sugar Test:

RANDOMISATION		Mean	Std. Deviation	Sig. value	Mean	Std. Deviation	Sig. value
		Standard			TFN		
Pair 1	RBS-BASELINE	153.5667	23.80189	.342	151.1000	19.40556	.479
	RBS-FOLLOW UP	156.3333	26.20389		150.8000	22.58837	

From the study specific laboratory investigation indicating the overall significance observed in the case of both the groups from randomization. The mean ranges of Free T3, Free T4, TSH,TT3 & TT4 was considerably improved from the baseline period after the TFN-32 treatment, thereby indicating the overall significance (p< .001)associated with their overall efficacy in regards to moderating the hormonal levels of thyroid hormones.

Study Specific Laboratory investigation:

Specific Laboratory investigation						
RANDOMISATION		RANDOMISATION		TOTAL	Chi-square	p-value
		Standard	TFN			
T3_BASELINE	Normal	14	8	22	2.584	.108
	Abnormal	16	22	38		
	Total	30	30	60		
T3-FOLLOWUP	Normal	7	23	30	17.067	<.001
	Abnormal	23	7	30		
	Total	30	30	60		
T4-BASELINE	Normal	19	17	36	.278	.598
	Abnormal	11	13	24		
	Total	30	30	60		

T4-FOLLOWUP	Normal	12	30	42	25.714	<.001
	Abnormal	18	0	18		
	Total	30	30	60		
TT3-BASELINE	Normal	9	12	21	.659	.417
	Abnormal	21	18	39		
	Total	30	30	60		
TT3-FOLLOWUP	Normal	18	29	47	11.882	<.001
	Abnormal	12	1	13		
	Total	30	30	60		
TT4-BASELINE	Normal	11	1	12	10.417	.001
	Abnormal	19	29	48		
	Total	30	30	60		
TT4-FOLLOWUP	Normal	4	26	30	32.267	<.001
	Abnormal	26	4	30		
	Total	30	30	60		
TSH-BASELINE	Normal	2	0	2	2.069	.150
	Abnormal	28	30	58		
	Total	30	30	60		
TSH-FOLLOWUP	Normal	1	4	5	1.964	.161
	Abnormal	29	26	55		
	Total	30	30	60		

From Zulewski scoring, the patients were categorized into 3 equal groups on the bases of thyroid function status into euthyroidisim, intermediate, hypothyroidism, and hyperthyroidism groups. The analyses showed a significant difference in all the parameters between the three groups except the platelets which showed no significant correlation. The primary outcome of this clinical trial was reduction of hypothyroidism symptoms followed with time taken by patients who were treated with Ayurvedic medicines in testing negative in the TSH, TT3, TT4, indicating expedited recovery in the treatment group. By the follow-up period of the 6th month showed that patients in the treatment group tested majority with negative symptoms from the scoring compared with only minimal percentage of patients exhibiting same outcome in the placebo group. Treatment group witnessed 100% recovery by the end of the 6 months followup period, while it was minimal compared to placebo group.

From the chi-square investigation on the subclinical and clinical hypothyroidism condition and their treatment approach involving TFN-32 and placebo showed that both the groups significantly improved their hypothyroidism condition as they were categorized as euthyroid and also intermediate from hypothyroidism condition during their baseline assessment. However compared between both the groups, TFN-32 showcased considerable efficacy, as they exhibited 100% recovery with patients were under euthyroid category by the end of 6th month followup.

CHI-SQUARE- Sub clinical and clinical hypothyroidism

Crosstab						
Crosstab						
		RANDOMISATION		Total	Chi-square	p-value
		Standard	TFN			
TOTAL SIGNS PRESENT WITH SCORE-ON ENTRY	Intermediate	1	6	7	4.043	.044
	Hypothyroid	29	24	53		
Total		30	30	60		
TOTAL SIGNS PRESENT WITH SCORE-2ND MONTH	Euthyroid	1	8	9	7.277	.026
	Intermediate	16	15	31		
	Hypothyroid	13	7	20		
Total		30	30	60		
TOTAL SIGNS PRESENT WITH SCORE-4TH MONTH	Euthyroid	12	22	34	7.608	.022
	Intermediate	16	8	24		
	Hypothyroid	2	0	2		
Total		30	30	60		
TOTAL SIGNS PRESENT WITH SCORE-6TH MONTH	Euthyroid	26	30	56	4.286	.038
	Intermediate	4	0	4		
Total		30	30	60		

Discussion:

Hypothyroidism and its related symptomatic abnormalities are common among population and is even more commoner when it comes to females from majority of the literature studies, as the current study comprised with female population alone (100%) similarly to most articles such as Dorgalaleh et al., (2020). In our study we divided the enrolled individuals in to 2 equal groups, comprised with each group included 30 individuals, the first group were cases comprised of hypothyroidism patients who were treated with placebo, and the other groups were those having hyperthyroidism who were administered with TFN-34, as these 2 groups compared with apparently healthy individuals as the control group, generally it is estimated that cases of hypothyroidism are higher than hyperthyroidism when cases are taken randomly (Kamdar et al., 2020).

With regards to the initial biochemical investigation for liver function test, renal function test and blood sugar levels from both the groups were found to be similar, however with much better outcomes identified in the case of TFN-34 compared to the placebo groups. These biochemical investigation holds significance as the study conducted by Ahmed & Mohammed (2020) which concluded similar outcomes and the level of haemoglobin was significantly associated with thyroid dysfunction (P value 0.000) followed with anaemia was present in 31.3% of the patients in this study.

Liver abnormalities in thyroid diseases or thyroid abnormalities in liver diseases were known in the Past. However, the cause and effect relationship between the two is now becoming clear. In the present study an attempt is made to assess liver functions (AST, ALT and ALP) in patients with subclinical and overt hypothyroidism and compare with healthy euthyroid controls. Our data showed a significant increase in AST, ALT and ALP levels in hypothyroid patients when compared to healthy controls and this increase was also significant when overt hypothyroid patients were compared with subclinical hypothyroid patients.

TSH Normally, low levels (less than 5 units) of TSH are sufficient to keep the normal thyroid gland functioning properly. When the thyroid gland becomes inefficient, such as in early hypothyroidism (Subclinical hypothyroidism), the TSH becomes elevated even though the T4 and T3 may still be within the "normal" range (Soldin et al., 2007). This rise in TSH represents the pituitary glands response to a drop in circulating thyroid hormone; it is usually the first indication of thyroid gland failure. Since TSH is normally low when the thyroid gland is functioning properly, the failure of TSH to rise when circulating thyroid hormones are low is an indication of impaired pituitary function. The new "sensitive" TSH test will show very low levels of TSH when the thyroid is overactive (as a normal response of the pituitary to try to decrease thyroid stimulation) (Ryan, 2017).
Interpretations of the TSH level depend upon the level of thyroid hormone; therefore, the TSH is usually used in combination with other thyroid tests such as the T4 and T3.T4 circulates in the blood in two forms. In one form, the T4 bound to proteins that prevent the T4 from entering the various tissues that need thyroid hormone.Free T4, which does enter the various target tissues to exert its effects. The free T4 fraction is the most important to determine how the thyroid is functioning, and tests to measure this are called the Free T4 (FT4) and the Free T4 Index (FT4I or FTI). Individuals who have hypothyroidism will have a low level of FT4 or FTI.Combining the TSH test with the FT4 or FTI accurately determines how the thyroid gland is functioning. The finding of an elevated TSH and low FT4 or FTI indicates primary hypothyroidism due to disease in the thyroid gland. A low TSH and low FT4 or FTI indicates hypothyroidism due to a problem involving the pituitary gland. The T4 reflects the amount of thyroxin in the blood.
T3 represents the active form of thyroid hormones. It increases cardiac output, cardiac contractility and heart rate and decreases systemic vascular resistance. T3 testing rarely is helpful in the hypothyroid patient, since it is the last test to become abnormal. Thyroxin (T4) represents 80% of the thyroid hormone produced by the normal gland and generally represents the overall function of the gland. The other 20% is T3. Patients can be severely hypothyroid with a high TSH and low FT4 or FTI, but have a normal T3. In some situations, such as during pregnancy or while taking birth control pills, high levels of total T4 and T3 can exist. This is because the estrogens increase the level of the binding proteins. In these situations, it is better to ask both for TSH and free T4 for thyroid evaluation. Sometimes the diseased thyroid gland will start producing very high levels of T3 but still produce normal levels of T4. Therefore measurement of both hormones provides an even more accurate evaluation of thyroid function. The T3/T4 ratio is a simply calculated index reflecting thyroid function and the action of hormones on the tissues. T3/T4 ratio is increased in hypothyroidism because of the higher efficiency of the thyroid gland to secrete the more biologically active fraction of the hormonesand the increased 5'-deiodinase tissue's activity. Additionally, in hypothyroidism T3/T4 ratio is much higher because of the pronounced decline of T4 levels. The ratio of serum free T3 (FT3) over free T4 (FT4) (FT3/FT4 ratio) may reflect the degree of extra thyroidal T4 to T3 conversion activity In thyroid dysfunction, the f T3/fT4 ratio changes owing to differently affected thyroidfunction and perpheral deiodination.

When considering renal function test, it was reported by authors that h**ypothyroid condition could be associated to significant derangement in biochemical parameters of renal function**. Hence the renal function should be

regularly monitored in hypothyroid patients.In a study which was done by Takahashi et al., (1990) who investigated different causes of anemia including macrocytic anemia, they found that hypothyroidism was one of the most important cause of anemia and abnormal red blood cell size .

Similarly,from an investigation conducted findings showcased 154 of 207 (74.4%) with Zulewski's score of ≥5, had deranged TSH. Overt hypothyroidism was seen in 62.34% (96 of 154) patients, and biochemical subclinical hypothyroidism was seen in 37.66% (58 of 154) patients. This was in concordance with the previous study by Zulewski et al., in which 62% of biochemically over hypot From Zulewski scoring, the patients were categorized into 2 equal groups on the bases of thyroid function status into euthyroidisim, intermediate, hypothyroidism, and hyperthyroidism groups. The analyses showed a significant difference in all the parameters between the two groups except the platelets which showed no significant correlation. The primary outcome of this clinical trial was reduction of hypothyroidism symptoms followed with time taken by patients who were treated with Ayurvedic medicines in testing negative in the TSH, TT3, TT4, indicating expedited recovery in the treatment group. By the follow-up period of the 6^{th} month showed that patients in the treatment group tested majority with negative symptoms from the scoring compared with only minimal percentage of patients exhibiting same outcome in the placebo group. Treatment group witnessed 100% recovery by the end of the 6 months followup period, while it was minimal compared to placebo group.

The main limitations of this study is that larger number of patients are required to increase the accuracy of the findings and some population based data are required to determine the normal geographical and age related variation regarding the levelof thyroid in the blood stream and the resultant variation in the outcomes achieved for the test parameters for monitoring hypothyroidism during the treatment.

Conclusion:

The study succeeded in conducting a detailed double blind randomized placebo controlled investigation that presented the safety and efficacy governing with the administration of TFN34 tablets for Individuals with subclinical & clinical hypothyroidism as assessed by clinical and laboratory methods including Zulewski Index questionnaire. The Serum FT3 level, FT4 level, TSH level - incidence was identified with better outcomes with no adverse events observed in the study. The clinical trial exhibited promising outcome with utilizing the TFN-34 for it efficacy in the treatment of hypothyroidism.

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