

Effect of a 6-month yoga intervention on heart rate variability among pre-diabetics

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ABSTRACT

Objectives: Pre-diabetes represents the initial stage of type 2 diabetic disease. This study aimed to highlight the importance of the 6-month integrated approach of yoga therapy (IAYT) on the time domain and frequency domain of heart rate variability (HRV) in pre-diabetic subjects.

Method: The study was conducted on 250 pre-diabetic adults aged 30–50 years attending a tertiary care hospital. The patients were divided into two groups: The study group (n = 125) was subjected to the IAYT and the control group (n = 125) was not engaged in yoga therapy. Biochemical parameters such as blood glucose, glycated hemoglobin, and the time domain and frequency domain of HRV were recorded at baseline and after 6 months of yoga intervention.

Results: Participants had a mean age of 45.4 ± 6.4 years, the post-yoga intervention resulted in a significant decline in blood glucose, glycated hemoglobin, and significantly decreased frequency domain parameters low frequency (LF), and LF/high-frequency ratio (LF/HF ratio), and significantly increased in high frequency (HF) and in time-domain parameter standard deviation of normal-normal (NN) interval, mean percentage of differences higher than 50 ms in RR intervals (pRR50), and root mean square of successive differences between normal heartbeat (RMSSD). The number of pairs of successive NN (R-R) intervals that differ by more than 50 ms (NN50) significantly increased.

Conclusion: The study clearly indicates that after 6 months of yoga intervention, autonomic nervous system shifted toward parasympathetic dominance, which was assessed by time domain and frequency domain parameters of HRV.

Keywords: Autonomic nervous system, frequency domain analysis, heart rate variability, time-domain analysis

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Introduction

Pre-diabetes is presented as a state of intermediate hyperglycemia where the levels of glucose in the blood are beyond the normal range (100–125 mg/dl) but below the diabetic range (>125 mg/dl), and mostly the diabetic subjects manifest the state of pre-diabetes before the onset.^[1]

Heart rate variability (HRV) changes the time interval between heartbeats. It is controlled by the autonomic nervous system, which also regulates many other vital body functions. HRV has been used as a non-invasive marker of cardiac autonomic activity and cardiovascular risk stratification.^[2] This system has two essential components, the sympathetic and parasympathetic systems. In a simplified sense, the sympathetic component increases heart rate and the parasympathetic part decreases heart rate. Thus, the observed HRV is an indicator of the dynamic

interaction and balance between these two components of the system.^[3]

HRV and heart rate turbulence were measured by Holter electrocardiogram (ECG), which was the most widely used method for the estimation of cardiac autonomic functions.^[4] HRV is defined as the distinction in period interlude among two consecutive heartbeats. It is a non-invasive assessment of the autonomic nervous system's sympathetic and parasympathetic limb of the autonomic nervous system.^[5] HRV has been a widely used technique used to measure the status of the autonomic nervous system.

Cardiovascular autonomic dysfunction is caused by the destruction of nerves and vessels innervating the heart and can drive cardiovascular dysfunction and vascular dynamics abnormality. Cardiovascular autonomic dysfunction is a

common complication of diabetes mellitus presented with arrhythmia, myocardial infarction, and sudden death.^[6] Symptoms of autonomic dysfunction include sinus tachycardia, exercise intolerance, myocardial ischemia, and orthostatic hypotension. One of the earliest markers of cardiac autonomic dysfunction is a reduction in HRV with parasympathetic loss that precedes sympathetic dysfunction.

In glucose regulation, homeostasis between sympathetic and parasympathetic components of the autonomic nervous system plays the principal role. Diabetic autonomic dysfunction is asymptomatic in 50% of patients and devastates an individual.^[7] Cardiac autonomic neuropathy is the prime reason for morbidity and mortality in diabetes. The current research has revealed the involvement of both limbs of the autonomic nervous system that is sympathetic and parasympathetic in type 2 diabetes.^[8] Several studies confirm the association between stress and the onset of diabetes and complications related to the disease.^[9]

HRV analyzes the characteristics of NN intervals. Linear parameters in HRV are divided into two categories: Time and frequency domains. HRV parameters of the time domain include the root mean square of the successive differences of NNs (RMSSD). The number of pairs of successive NNs that differ by more than 50 ms divided by the total number of NNs (pNN50)^[10] while those of frequency domain include spectral power of NNs in high frequency (HF) (HF, 0.15–0.4 Hz) reflecting parasympathetic activity, low frequency (LF) (LF, 0.04–0.15 Hz) indicating sympathetic function, and LF-to-(HF) ratio (LF/HF) as an index of autonomic balance.^[11]

Lifestyle interventions such as yoga, an ancient practice that is said to benefit all components of health, may prove to be helpful in non-pharmacological interventions in stopping the progression of pre-diabetes from type 2 diabetes and related complications.^[12]

Yoga involves a wide range of mind-body practices such as meditation, relaxation techniques, pranayama, and asanas/postures that integrate the mind and body homeostasis and help in physical, mental, intellectual, and spiritual development. Khalsa^[13] reported that yoga practices reduce autonomic arousal and assist with a wide range of stress-related disorders. This may be mediated by increased parasympathetic activity as indicated by the increased high-frequency component of HRV. Howorka *et al.*^[14] and Pitale *et al.*^[15] reported decreased in frequency domain parameters, LF, and increased HF and time domain parameters standard deviation of normal (SDNN), mean percentage of differences higher than 50 ms in RR intervals (pRR50), and root mean square of successive differences between normal heartbeat (RMSSD). The number of pairs of successive NN (R-R) intervals that differ by more than 50 ms (NN50) significantly increased after yoga intervention. Innes *et al.*^[16] reported associations between yoga and markers of autonomic activity such as heart rate,

Bowman *et al.*^[17] observed a positive correlation between yoga and baroreflex sensitivity, and Telles *et al.*^[18] reported yoga intervention improved galvanic skin resistance in the yoga group compared to the control group. Tyagi^[19] and Cohen reported that regular yoga practice improves a wide range of clinical conditions associated with autonomic dysfunction, such as hypertension and diabetes.

An integrated approach yoga therapy (IAYT) approach includes practicing yoga, controlled breathing, dietary modification, and counseling. Early diagnosis of altered cardiac autonomic functions and arterial stiffness can lead to an effective strategy for the treatment of pre-diabetic conditions and reduce cardiovascular risk. Therefore, this study plans to clarify the IAYT and its association with time and frequency domain parameters of HRV among pre-diabetics.

Methods

The present study was designed to assess and correlate the effect of the IAYT on time and frequency domain parameters of HRV among pre-diabetics. The study was designed as experimental interventional study, conducted in the Research Lab Department of Physiology, Rajasthan University of Health Sciences, Jaipur, after obtained permission from the RUHS Institutional Ethics Committee (Registration no. ECR/762/Inst/RJ/01). A random sample of the population in RUHS college of medical sciences and those attending outpatient departments at the institute through hospital-based advertisements, flyers, face-to-face contact, and word-of-mouth were selected for the screening of fasting blood glucose for the study. After the screening of 2000 subjects, a total of 250 pre-diabetic subjects were recruited. Randomization and allocation in the study and control groups were performed using computer-generated random numbers. After the explanation of the study objectives and the importance of an IAYT verbally and in the participation information sheet, subjects, who gave written informed consent were included in this study.

Subjects who voluntarily enrolled in the IAYT and controls were included in the study. The sample size was calculated using the appropriate formula z^2pq/d^2 , where p and q were taken as 0.08 and 0.92 to get the maximum sample size with a 5% permissible error (precision) and 10% non-response rate. The sample size was 250 with a 95% confidence interval.^[3] Participants included a fasting glucose level of 110 levels mg/dL and glycated hemoglobin 5.7–6.4 (ADA criteria),^[1] no history of cardiovascular disease, and no history of diabetic medications. Subjects who have fasting blood glucose <100 mg/dl and >126 mg/dl, renal dysfunction, liver disease, alcoholic individuals, retinopathy and neuropathy, interstitial fibrotic disease, spinal injury, and those being treated with anti-inflammatory medication were excluded from the study.

The IAYT included prayer, Omkar recitation, yoga postures that included sukhasana, bhujangasana, suryanamaskar

pashimottanasana, tadasana, padmasana, trikonasana, ardhmatsyendrasana, sarvangasana, pawanmuktasana, vajrasana, dhanurasana, shavasana, breathing (pranayama) techniques, Shavasana counseling, and diet (vegetables, fruits, legumes, and whole grains). Yoga asanas were supervised and demonstrated by a certified yoga instructor. Yoga sessions were approximately 45 min 6 days per week for 6 months. To facilitate home practice, participants were given video clips of the yoga asanas recorded under the direction of the certified yoga instructor, and the compliance of subjects was checked by daily messages on WhatsApp and weekly telephonic conversions with subjects and family members. The evaluation was done at baseline, 3 and 6 months of post-intervention.

Analysis of HRV – The electrodes were placed on the pressure pad of the finger pulse transducer. The HRV analysis in the frequency domain reflects the speed variation in heart rate. Further, this method also gives information about different frequency components of the N-N intervals and their power or variance. Digital physiograph AD instruments recorded ECG signals. The signals were filtered digitally and processed to extract QRS peaks which determine the R-R intervals. These QRS peaks were automatically detected and were reviewed visually for R-wave determination and ectopic beats. Areas of ECG in which identification of moments was poor or ectopic beats were present were excluded from the study. The time and frequency domain indices were computed from 5 min segments.^[20]

Linear dynamics

Time domain analysis

The time domain indices computed using statistical methods on RR tachogram included mean-mean of RR interval (RR), the standard deviation of RR interval SDNN, the square root of the mean of the sum of the squares of the differences between adjacent RR intervals (RMSDD), and the percentage of RR 50 counts, given by RR 50 count divided by the total number of all RR intervals (pRR50). Among these indices from linear dynamics of short-term HRV, SDNN, RMSDD, and pRR50 represent the cardiac parasympathetic drive (vagal tone).^[2]

Frequency domain analysis

Frequency domain analysis was done by power spectral analysis using fast Fourier transformation. The frequency domain indices included LF (LF; 0.04–0.15 Hz), (HF) (HF; 0.15–0.4 Hz), and the ratio of LF to HF (LF-HF ratio).^[2]

Statistical analysis of data

Mean and standard deviations were calculated for each parameter. The appropriate tool for comparing the change in the level of a variable is the student's paired t-test for intragroup comparison. Before applying this test, the Smirnov–Kolmogorov test was performed to assess the normality of each parameter.

Apart from comparing the various data, parameters concerning before and after yoga sessions. In the study and control group, 125 subjects were in each group. At the baseline, all the subjects in the study and the control group were pre-diabetics. Student paired t-test was conducted for intragroup comparison and one-way analysis of variance (ANOVA) was conducted for intragroup comparison at baseline, 3 and 6 months. $P > 0.05$ was considered as non-significant.

Results

Table 1 depicts the age and gender distribution of subjects; majority of subjects were female and age group of 41–50 years. The mean age was 42.1 ± 3.42 .

Table 2 depicts the mean values of blood glucose and glycated hemoglobin in the control and study groups at baseline, 3 months, and 6 months. Intragroup comparison of blood glucose and glycated hemoglobin in the control and study groups was done by one-way ANOVA for measures taken. Significance level tested by *post hoc* test Tukey's test and Scheffe's test. After 6 months, blood glucose and glycated hemoglobin significantly decreased in the study group and control group results were non-significant.

Table 3 shows the mean values of LF, HF, and LF/HF ratio in the control and study groups at baseline, 3 months, and 6 months. Intragroup comparison of HRV in the control and study groups was done by one-way ANOVA for measures taken at different times. Significance level tested by *post hoc* test Tukey's test and Scheffe's test. After 6 months, LF power spectrum and LF/HF ratios significantly decreased and HF power spectrum significantly increased in the study group and control group results were non-significant.

Table 4 depicts the intragroup comparison of mean values of time domain indexes (SDNN, RMSDD, pRR50, and NN 50) in the control and study groups at baseline, 3 months, and after 6 months. One-way ANOVA for measures was taken at different times. Significance level tested by *post hoc* test Tukey's test and Scheffe's test. Time domain variables such as SDNN, RMSDD, pRR50, and NN 50 significantly increased in the study group and in the control group results were non-significant.

Table 5 depicts Pearson correlation analysis between frequency domain parameters (LF/HF ratio) blood glucose, glycated hemoglobin, and time domain parameters (RMSDD, pRR50, and NN50) in the control and study group. After 6 months of

Table 1: Age and gender distribution of the study population

Age group	Male	Female	Total
30–40 years	25	50	75
41–50 years	75	100	175
Total	100	150	250

Table 2: Analysis of variance analysis for intragroup comparison of biochemical parameters (blood glucose and glycated hemoglobin)

Parameters	Groups	Baseline	3 months	After 6 months	P-value
		Mean±SD	Mean±SD	Mean±SD	
Blood glucose	Control	116.87±4.57	117.66±4.95	119.45±4.88	0.799
	Study	116.87±4.57	108.75±5.51	100.20±3.38	<0.0001
Glycated hemoglobin	Control	6.40±0.66	6.42±0.80	6.42±0.45	0.697
	Study	6.42±0.86	5.67±0.40	5.17±0.10	<0.01

SD: Standard deviation

Table 3: Analysis of variance analysis for intragroup comparison of frequency domain parameters (LF, HF, and LF/HF ratio of heart rate variability)

Parameters	Groups	Baseline	3 months	After 6 months	P-value
		Mean±SD	Mean±SD	Mean±SD	
LF	Control	65.72±11.44	66.72±9.88	67.82±12.44	0.676
	Study	66.67±11.87	45.67±13.9	34.65±11.6	<0.001
HF	Control	45.90±11.79	43.77±7.89	40.75±11.77	0.899
	Study	36.40±11.75	51.56±13.06	65.44±15.5	<0.0001
LF/HF ratio	Control	2.18±1.09	2.16±1.04	2.20±1.05	0.982
	Study	2.14±1.09	1.05±13.06	0.57±0.54	<0.0001

LF: Low frequency, HF: High frequency, LF/HF ratio: Low-to-high-frequency ratio

Table 4: Analysis of variance for intragroup comparison of time domain parameters (SDNN, RMSDD, PRR50, and NN50) of heart rate variability

Parameters	Groups	Baseline	3 months	After 6 months	P-value
		Mean±SD	Mean±SD	Mean±SD	
SDNN	Control	33.47±3.95	31.22±3.15	35.45±3.45	0.645
	Study	33.28±3.88	43.98±4.81	59.07±4.77	<0.001
RMSDD	Control	22.27±2.95	21.22±2.15	22.35±2.45	0.896
	Study	31.28±2.88	33.98±2.91	61.07±4.38	<0.001
PRR50	Control	14.67±1.76	13.85±1.86	14.5±1.55	0.765
	Study	17.36±2.25	25.67±2.61	77.53±4.19	<0.0001
NN50	Control	26.50±2.95	25.50±2.66	26.85±2.45	0.588
	Study	24.50±4.95	35.66±3.61	55.66±3.61	<0.0001

SDNN: Standard deviation of normal-to-normal RR intervals, RMSDD: Root mean square of successive differences between normal heartbeat, PRR50: Percentage of differences higher than 50 ms in RR interval, NN50: The number of pairs of successive NN (R-R) intervals that differ by more than 50 ms

IAYT, a significant positive correlation was found between frequency domain parameters (LF/HF ratio) and time domain parameters (RMSDD, pRR50, and NN50), and a significant negative correlation was found between LF/HF ratio and blood glucose and glycated hemoglobin.

Discussion

Studies on the linear component of time domain and frequency domain of HRV among pre-diabetics after 6 months of the IAYT program are not well documented. Hence, in this study, an attempt has been made to assess linear parameters – time domain and frequency domains among pre-diabetics and establish a correlation between the blood glucose, glycated hemoglobin, time, and frequency domain parameters of HRV.

HRV was a powerful tool for investigating the effect of ANS on the cardiovascular system. Autonomic dysfunction can adversely affect heart function. HRV can serve as a sensitive method of diagnosing early changes in cardiac autonomic functions.^[11,12]

In the study group, after 6 months of yoga intervention significantly decreased the biochemical parameters such as blood glucose (116.87 ± 4.85 – 100.75 ± 3.38 , $P < 0.001$) and glycated hemoglobin (6.42 ± 0.86 – 5.17 ± 0.10 , $P < 0.01$) and decreased frequency domain parameters, LF power spectrum (66.67 ± 11.87 – 34.65 ± 11.6 ; $P < 0.001$), and LF/HF ratio (2.14 ± 1.09 – 0.57 ± 0.54 ; $P < 0.0001$) and a significant increase in HF power spectrum (36.40 ± 11.75 – 65.44 ± 15.5 ; $P < 0.001$) and increased in time domain parameters, SDNN (33.28 ± 3.88 – 59.07 ± 4.77 ms, $P < 0.001$) RMSDD (31.28 ± 2.88 – 61.07 ± 4.38 ms, $P < 0.001$), PRR50 (17.36 ± 2.25 – 77.53

Table 5: Pearson correlation analysis between frequency domain parameters (LF/HF ratio) and blood glucose, glycated hemoglobin (HbA1C), and time domain parameters (SDNN, RMSDD, PRR50, and NN50)

Parameters	Frequency domain parameters							P-value
	Time domain parameters	Groups	LF/HF ratio				P-value	
			Baseline		3 months			
		Pearson correlation	Sig. (two tailed)	Pearson correlation	Sig. (two tailed)	Pearson correlation	Sig. (two tailed)	
Blood glucose	Control	0.044	0.288	0.069	0.488	0.043	0.346	NS
	Study	0.068	0.351	-0.532	0.01	-0.922	0.001	**
HbA1C	Control	0.039	0.276	0.067	0.634	0.054	0.754	NS
	Study	0.057	0.452	-0.589	0.01	-0.688	0.01	*S
SDNN	Control	0.044	0.381	0.064	0.488	0.084	0.580	NS
	Study	0.082	0.180	0.455	0.01	0.0766	0.01	**
RMSDD	Control	0.034	0.481	0.054	0.599	0.097	0.280	NS
	Study	0.097	0.280	0.432	0.01	0.988	0.001	**HS
PRR50	Control	0.058	0.481	0.067	0.577	0.089	0.754	NS
	Study	0.067	0.390	0.466	0.01	0.856	0.001	**HS
NN50	Control	0.064	0.288	0.035	0.476	0.097	0.280	NS
	Study	0.077	0.356	0.598	0.01	0.705	0.01	**HS

$P < 0.05$, S*: Significant, HS**: Highly significant, NS: Non-significant, HbA1C: Glycated hemoglobin, LF/HF ratio: Low-frequency/high-frequency ratio, SDNN: Standard deviation of normal-to-normal RR intervals, RMSDD: Root mean square of successive differences between normal heartbeat, PRR50: Percentage of differences higher than 50 ms in RR interval, NN50: The number of pairs of successive NN (R-R) intervals that differ by more than 50 ms

± 4.19 ms, $P < 0.0001$), and NN50 (24.50 ± 4.95 – 55.66 ± 3.61 ms, $P < 0.0001$), and in the control group results were non-significant.

Vaishali *et al.*^[21] reported in a yoga intervention study conducted on type 2 diabetes subjects conducted over a period of 3 months and observed that post-yoga there was a significant decrease in blood glucose (163.45 ± 14.8 – 115.62 ± 13.7 ; $P < 0.001$) and glycated hemoglobin levels (10.28 ± 0.86 – 9.12 ± 0.55 ; $P < 0.01$) in the study group results were similar to the present study.

Meshram and Meshram^[22] reported in a study that after 6 months of yoga intervention revealed that in the frequency domain parameters, significantly decreased low-frequency (LF) power spectrum ($59.12 + 3.65$ – $56.90 + 3.57$), LF/HF ratio ($2.49 + 0.18$ – $2.19 + 0.19$) and significantly increased in high-frequency power spectrum (HF) ($23.7 + 1.55$ – $26.0 + 2.15$) and in time domain parameters significantly increased SDNN ($24.06 + 2.01$ – $26.11 + 1.90$), RMSDD ($23.88 + 2.15$ – $25.34 + 2.11$), and PNN50 ($2.48 + 0.21$ – $2.96 + 0.24$) similar to the present study.

Vinay *et al.*^[23] observed in a yoga intervention study conducted over a period of 1-month post-yoga analysis of HRV revealed that in the frequency domain parameters, significantly decreased in the LF power spectrum from 39.30 to 30.40 and LF/high-frequency ratio from 2.62 to 2.28 and in time domain parameters, significantly increased in SDNN from 33.60 to 42.11, RMSDD from 22.00 to 25.6, and PNN50 increased from 2.45 to 7.3 results were similar to the present study.

Kuppusamy *et al.*^[24] reported that after 6 months of yoga breathing practices, significantly decreased frequency domain parameters LF (65.06 ± 11.67 – 57.02 ± 12.29), LF/HF ratio (1.60 ± 0.42 – 1.37 ± 0.40), and increased HF power spectrum (40.08 ± 9.01 – 46.87 ± 11.22) and significantly increased the time domain parameters such as SDNN (80.03 ± 10.14 – 88.06 ± 14.83), RMSDD (59.91 ± 13.50 – 62.05 ± 16.93), NN50 (26.29 ± 10.16 – 29.68 ± 11.02), and pNN50 (26.29 ± 10.16 – 29.68 ± 11.02) results were similar to the present study.

Vinutha *et al.*^[25] reported in a study conducted on a total of 15 diabetic patients after 7 days of yoga intervention, that there was a significant reduction in fasting plasma glucose from 154.67 to 130.27 mg/dL, $P < 0.01$. The increase in the high-frequency power spectrum (174.38 ± 214.33 – 185.34 ± 144.69), RMSDD (16.71 ± 9.20 – 17.84 ± 7.75), and PRR50 (1.89 ± 5.28 – 2.08 ± 3.69) results was similar to the present study.

Hautala *et al.*^[26] and Billman and Kukielka^[27] reported that after yoga intervention significant decrease ($P < 0.05$) in the LF component and LF/HF ratio and a significant increase ($P < 0.05$) in the HF component, which represents the shifting of the autonomic nervous system in favor of parasympathetic branch similar to the present study.

In this present study, yoga practice over 6 months significantly reduced the time domain and frequency domain parameters. It can be attributed to the inhibition of the posterior sympathetic area of the hypothalamus that enhances body sympathetic responses to stress conditions. This assists in the homeostasis of autonomic regulatory reflex and reflects autonomic balance,

suggesting parasympathetic dominance in the autonomic nervous system.^[28]

The lifestyle modification strategy is the first line of treatment before pharmacological management to mitigate the development of pre-diabetes to diabetes.

Limitation

The major limitations of the present study are the small sample size and conducted in a single center. The other limitation is the short duration of the intervention. In the future, randomized controlled trials with large sample sizes and multicentric could be studied. Studying the effect of the long-term practice of yoga on autonomic functions in pre-diabetes patients would be helpful.

Conclusion

This study depicts that after 6 months of yoga intervention significant decrease in blood glucose, glycated hemoglobin, time domain, and frequency domain parameters of HRV. Regular yoga practice increases vagal tone and improves the autonomic functions in the study group compared to the control. HRV, therefore, reflects the mind-body integration that occurs with many yoga practices by directly linking the input and output of the central nervous system. It indicates that yoga can decrease the risk of pre-diabetes and prevent the progression of pre-diabetes to diabetes. Therefore, yoga programs could be an interventional approach to decrease cardiovascular risk and increase exercise efficacy in the pre-diabetic group. The ease of use, safety, and multiple psychological benefits of yoga have led it to be widely accepted in society, and it can now be considered a low-cost non-pharmacological intervention to control various lifestyle disorders, including pre-diabetes, diabetes, and cardiovascular disease resulting in significant positive clinical outcomes. Finally, multicentric, cost-effectiveness, and long-term follow-up studies are lacking. All of these areas warrant future research. Additional high-quality investigations are required to confirm and further elucidate the potential therapeutic benefits of yoga programs among pre-diabetes for primary prevention of diabetes.

Authors' Declaration Statements

Ethics approval and consent to participate

This study was approved by the RUHS Institutional Ethics Committee (Registration no. ECR/762/Inst/RJ/01). The participants received explanations of the purpose and contents of the study, the voluntary nature of their participation, and the benefits of this yoga practice in the participant information sheet. The written informed consent form was then obtained from each participant parents.

Consent for publication

I understand my rights and my responsibilities as a participant in this study. I permit the investigator to utilize my information and the results obtained from this study for presentation and publication (participant language).

Data availability statement

The data that support the findings of this study will be made available from the corresponding author, on request.

Competing interests

All authors declare no competing interests.

Funding statement

Authors declare no external funding for this research.

Authors' Contributions

1. Sudhanshu Kacker: Designing the study, specifying the research question, data validation, editing, and checking the manuscripts drafts and final manuscript
2. Neha Saboo: Obtaining ethics committee approval, and study participants screening and recruitment, collecting, analyzing, and interpreting data writing, reading, checking, and editing in the final manuscript.

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