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Original Article

Physiology Section

Comparison of Lipid Profile in Prediabetic and Non Prediabetic Adult Off-springs of Type 2 Diabetics Patients: A Cross-sectional Study

SAYALI EKNATHRAO RAUT[1], SMITA SURESH BUTE[2], URJITA ZINGADE[3], ATISH BHASKAR PAGAR[4]

ABSTRACT

Introduction: Lipid abnormality is an important modifiable risk factor associated with the type 2 diabetes mellitus and prediabetes. Dyslipidaemia occurring in diabetic patients, has important role in development of macrovascular atherosclerosis and increases the risk of cardiovascular disease. Furthermore, prediabetes has also been found to be associated with an increased risk for cardiovascular disease. Considering the prevalence and increased risk of cardiovascular disease in diabetes, it is becoming necessary to diagnose prediabetic individuals and assess their lipid profile and prevent them from developing overt diabetes and the further complications. Also, data available on lipid abnormalities in prediabetics is relatively less in the Indian population.

Aim: To compare lipid profile in prediabetic and non prediabetic adult off-springs of type 2 diabetics and to evaluate the association between lipid profile and prediabetes.

Materials and Methods: This cross-sectional study was conducted on 150 healthy young adult (>18 years) off-springs of type 2 diabetic patients, willing to participate in the study in Government Medical College and Hospital, Miraj from January 2019 to December 2019. All the relevant information was collected by administering a structured case record form. Fasting blood samples were collected and fasting blood glucose level, lipid profile including Total cholesterol (TC), Triglycerides (TG), Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL), Very Low-Density Lipoproteins (VLDL) were estimated and compared. Data collected was entered in the Microsoft Excel (2010), expressed as frequency and mean. Chi-square test and Fisher's-exact test was applied to observe the association between different study parameters. A p-value <0.05 was considered statistically significant.

Results: Prevalence of prediabetes was 17.3% (fasting BLS 100 to 125 mg/dL) in age group of 26 to 30 years (26.67%). It was found that occurrence of prediabetes was more in male participants (25.37%) as compared to female participants (10.84%). Association between gender of the participants and occurrence of prediabetes was found to be statistically significant (p-value=0.019). The association between prediabetes and higher TC levels, lower HDL levels, higher LDL levels, higher VLDL levels was found to be statistically significant (p-value <0.05) and the association between prediabetes and higher TG levels (mean-48.65±18.45 and 28.18±9.47 mg/dL) was not significant (p-value=0.056).

Conclusion: Total cholesterol, LDL, TG, VLDL were significantly raised, whereas HDL was significantly lower in prediabetic subjects as compared to non prediabetic healthy subjects. The association between prediabetes and higher TC levels, lower HDL levels, higher LDL levels, higher VLDL levels was found to be statistically significant (p-value<0.05) and the association between prediabetes and higher TG levels was not significant (p-value=0.056). So, prediabetic individuals, though asymptomatic have significant dyslipidaemia, that puts them at higher risk for developing cardiovascular disease.

Keywords: High density lipoproteins, Low density lipoproteins, Prediabetes, Total cholesterol, Triglycerides, Very low density lipoproteins

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by chronic hyperglycaemia with deranged fat, carbohydrate and protein metabolism resulting from inadequate secretion or action of insulin [1]. It is a modern day epidemic. World Health Organisation (WHO) estimates that diabetes will become the seventh most common cause of mortality, worldwide in the year 2030 [2].

India is the diabetes capital of the world because there are around 41 million Indians suffering from diabetes till date and every fifth person in world, having diabetes, is an Indian [3,4]. Insulin resistance in children of type 2 diabetics is found to be 30% in siblings and 80% in identical twins [5]. It is difficult to diagnose early, as it is mostly asymptomatic and usually presents with complications like nephropathy, cardiovascular disease, retinopathy, neuropathy, cerebrovascular disease and peripheral vascular disease [6]. It can go undetected for 9-12 years and consequently, presents with complications [7]. Recent studies have revealed that around half of the diabetics in the world are undiagnosed [3,4].

American Diabetic Association has introduced a new category of blood glucose levels, preceding the onset of diabetes, known as prediabetes. Prediabetic individuals are at greater risk of development of diabetes [7]. According to American Diabetic Association, person is labelled as prediabetic, when fasting plasma glucose level ranges from 100 to 125 mg/dL and/or when plasma glucose level 2 hours after an oral glucose tolerance test ranges from 140 to 199 mg/dL [8].

Though, prediabetes is an asymptomatic condition, it is always present before the onset of diabetes. Rise in blood sugar level is linear process so, prediabetes is not an entirely harmless condition [8].

In diabetic patients, dyslipidaemia plays a critical role in development of macrovascular atherosclerosis leading to increase in the risk of cardiovascular disease. Likewise, prediabetes is also associated with an increased risk for cardiovascular disease [9]. As the prevalence of diabetes and so the risk of cardiovascular disease is increasing day-by-day, it has become important to diagnose prediabetic individuals, assess them for lipid profile and with early interventions prevent them from developing further complications.

The benefits of screening and treatment of lipid disorders in known type 2 diabetics with cardiovascular disorders are well documented. Still, there are no clear recommendations regarding screening for lipid disorders in asymptomatic prediabetic individuals. This is one

of few studies, seeing the association of lipid profile with prediabetes in adult off-springs of known cases of type 2 diabetics, so as to help to form future prevention programs for diabetes and related complications. The aim of the present study was to compare lipid profile in prediabetic and non prediabetic adult off-springs of type 2 diabetics and to evaluate the association between lipid profile and prediabetes.

MATERIALS AND METHODS

This cross-sectional study was carried out in 150 apparently healthy young adults (>18 years) off-springs of either gender of type 2 diabetic patients in Outpatient Department of General Medicine, Government Medical College and Hospital, Miraj, Maharashtra, India, from January 2019 to December 2019. Ethical committee clearance was obtained from Institutional Ethical Committee (Ref No. GMCM/E-C/24/2018 Dated 26/10/2018).

Inclusion criteria: Apparently healthy young adults (>18 years) off-springs of known cases of type 2 diabetics of either gender willing to participate in the study were included in the study.

Exclusion criteria: Subjects with type 1 and type 2 diabetes mellitus, prediabetics and on hypoglycaemic medicines, acute and chronic inflammatory disease, macular disorders, history of major illness or on treatment of renal disease, heart disease, hypoglycaemia, dyslipidemia, thyroid disorders, tuberculosis and pregnant women were excluded from the study.

Sample size calculation: In a study by Pandey U et al., [10], proportion of prediabetes among the study subjects was 32.1%. So according to the formula,

$$\text{Sample size } n=\frac{4\times P\times Q}{L^2}$$

P=Proportion=32.1%

Q=100-P=67.9%

L=Margin of error=10% (at 95% Confidence Interval)

$$n=\frac{4\times 32.1\times 67.9}{10\times 10}=87.18$$

n≈87

Though calculated sample size was 87, 150 study subjects were enrolled during the study period between January 2019 to December 2019.

Method of Collection of Data

Apparently healthy young adult off-springs of known cases of type 2 diabetes mellitus patients, accompanying their parents, willing to participate were included in the study. They were informed about the study and written informed consent was obtained from each participant and they were requested to come in fasting state (minimum 8 hours) when they come for next follow-up with their parents and blood sample were taken at that time or blood sample was collected by the investigator from their residence, as per the convenience of the participant. All the information was collected by administering a structured case record form.

Following symptoms of hyperglycaemia were enquired in all subjects included in the study,

1. Excessive thirst and drinking
2. Frequent urination
3. Recent weight loss
4. Fatigue
5. Recurrent thrush or skin infections

The blood samples were collected with all aseptic precautions by the investigator by venepuncture in non dominant arm in sitting position after fasting of minimum 8 hours and following parameters were measured.

a. Fasting Blood Sugar Level (fasting BSL): It was measured by enzymatic Glucose Oxidase Peroxidase method. Subjects were considered as prediabetic, if fasting BSL was between 100 to 125 mg% [11,12].

b. Lipid profile: Total cholesterol (TC), Triglycerides (TG), Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL), Very Low-Density Lipoproteins (VLDL), cholesterol/HDL were measured. Enzymatic method was used to measure total cholesterol and triglyceride levels. After centrifugation, the cholesterol in the HDL fraction, remaining in the supernatant was assayed with enzymatic Cholesterol Oxidase Peroxidase method. HDL-C was estimated after precipitation of chylomicrons. Whereas, VLDL and LDL fractions of cholesterol were measured using phosphotungstic acid and magnesium chloride. All the estimations were done with Transasia biochemistry fully autoanalyser- XL640.

Subjects, found to be diabetic or prediabetic were referred to Medicine OPD for further management.

STATISTICAL ANALYSIS

Data collected was entered in the Microsoft excel (2010). Continuous and basic characteristics of study subjects were displayed as frequency (percentage) and mean with Standard Deviation (SD). The association between different study parameters were assessed using Chi-square test. Especially when more than 20% of cells had expected frequencies <5, Fisher's exact test was used, because applying approximation method was inadequate. Unpaired t-test was used to compare lipid profile between prediabetics and healthy participants. A p-value was considered as significant, when <0.05.

RESULTS

In present study, out of 150 participants, 83 (55.3%) were females and 67 (44.7%) were males. Females were slightly more than males. Male to female ratio was 0.81:1.

[Table/Fig-1] shows that out of 150 participants, mostly, 65 (43.3%) were from age group 21 to 25 years followed by 34 (22.7%) were from age group ≤20 years and 31 (20.7%) from age group 31 to 35 years. Only 15 (10%) and 5 (3.3%) participants were from age group 26 to 30 years and >35 years respectively. Mean age of the participants was 25.01±5.55 years ranging from 18 to 36 years.

Age groups	Frequency	Percentage (%)
≤20	34	22.7%
21 to 25	65	43.3%
26 to 30	15	10.0%
31 to 35	31	20.7%
>35	05	3.3%
Total	150	100%

[Table/Fig-1]: Age-wise distribution of the participants.

[Table/Fig-2] shows classification of participants on the basis of fasting blood sugar level. Total 26 (17.3%) participants were found to be prediabetic and remaining 124 (82.7%) participants had normal fasting blood sugar levels.

Prediabetics	Frequency	Percentage
Yes (Fasting BSL 100 to 125 mg/dL)	26	17.3%
No (Fasting BSL <100 mg/dL)	124	82.7%
Total	150	100%

[Table/Fig-2]: Classification of healthy participants and prediabetic participants.

[Table/Fig-3] shows that occurrence of prediabetes was maximum in age group 26 to 30 years (26.67%) followed by 17.64% in age group ≤20 years but association between age of the participants and occurrence of prediabetes was not found to be statistically significant (p-value=0.749). Mean age of the prediabetics and healthy

participants were 25.62±1.70 and 25.65±1.75 years, respectively and the difference was not statistically significant (p-value=0.845).

Age groups	Prediabetes		Total
	Yes	No	
≤20 years	06 (17.64%)	28 (82.36%)	34 (100%)
21 to 25 years	11 (16.92%)	54 (83.08%)	65 (100%)
26 to 30 years	04 (26.67%)	11 (73.33%)	15 (100%)
31 to 35 years	05 (16.13%)	26 (83.87%)	31 (100%)
>35 years	0	05 (100%)	05 (100%)
Total	26 (17.3%)	124 (82.7%)	150 (100%)
Mean age (years)	25.62±1.70	25.65±1.75	p-value for mean age 0.845

[Table/Fig-3]: Association between age of the participants and prediabetes.
Fisher's exact test value=1.218, p-value= 0.749

[Table/Fig-4] shows that occurrence of prediabetes was more in male participants (n=17; 25.37%) as compared to female participants (n=9; 10.84%). Association between gender of the participants and occurrence of prediabetes was found to be statistically significant (p-value=0.019).

Gender	Prediabetes		Total
	Yes	No	
Male	17 (25.37%)	50 (74.63%)	67 (100%)
Female	9 (10.84%)	74 (89.16%)	83 (100%)
Total	26 (17.3%)	124 (82.7%)	150 (100%)

[Table/Fig-4]: Association between gender of the participants and prediabetes.
Chi-square=5.462, p-value=0.019

[Table/Fig-5] shows that out of 17 participants with TC >200 mg/dL, 10 (58.82%) participants were found to have prediabetes. Whereas out of 133 participants with TC ≤200 mg/dL, only 16 (12.03%) participants were found to have prediabetes. The association between prediabetes and higher TC levels was found to be statistically significant (p-value <0.05).

Total cholesterol	Prediabetes		Total
	Yes	No	
>200 mg/dL	10 (58.82%)	7 (41.18%)	17 (100%)
≤200 mg/dL	16 (12.03%)	117 (87.97%)	133 (100%)
Total	26 (17.3%)	124 (82.7%)	150 (100%)

[Table/Fig-5]: Association between Total Cholesterol (TC) levels of the participants and prediabetes.
Chi-square=23.03, p-value <0.05

[Table/Fig-6] shows that out of 28 participants with HDL ≤40 mg/dL, 11 (39.29%) participants were found to have prediabetes. Whereas out of 122 participants with HDL >40 mg/dL, only 15 (12.30%) participants were found to have prediabetes. The association between prediabetes and lower HDL levels was found to be statistically significant (p -value=0.001).

HDL	Prediabetes		Total
	Yes	No	
≤40 mg/dL	11 (39.29%)	17 (60.71%)	28 (100%)
>40 mg/dL	15 (12.30%)	107 (87.70%)	122 (100%)
Total	26 (17.3%)	124 (82.7%)	150 (100%)

[Table/Fig-6]: Association between high density lipoprotein of the participants and prediabetes.
Chi-square=11.58; p-value=0.001

[Table/Fig-7] shows that out of 5 participants with LDL >130 mg/dL, all 5 participants were found to have prediabetes. Whereas out of 145 participants with LDL ≤130 mg/dL, only 21 (14.48%) participants were found to have prediabetes. The association between prediabetes and higher LDL levels was found to be statistically significant (p-value <0.05).

Low density lipoprotein	Prediabetes		Total
	Yes	No	
>130 mg/dL	5 (100%)	0	5 (100%)
≤130 mg/dL	21 (14.48%)	124 (85.52%)	145 (100%)
Total	26 (17.3%)	124 (82.7%)	150 (100%)

[Table/Fig-7]: Association between low density lipoprotein of the participants and prediabetes.
Fisher's exact test value-24.67, p- value <0.05

[Table/Fig-8] shows that out of 69 participants with VLDL >30 mg/dL, 26 (37.68%) participants were found to have prediabetes. Whereas out of 81 participants with VLDL ≤30 mg/dL, none of the participants was found to have prediabetes. The association between prediabetes and higher VLDL levels was found to be statistically significant (p-value <0.05).

Very low density lipoprotein	Prediabetes		Total
	Yes	No	
>30 mg/dL	26 (37.68%)	43 (62.32%)	69 (100%)
≤30 mg/dL	0	81 (100%)	81 (100%)
Total	26 (17.3%)	124 (82.7%)	150 (100%)

[Table/Fig-8]: Association between very low density lipoprotein of the participants and prediabetes.
Fisher's exact test value=36.92, p-value <0.05

[Table/Fig-9] shows that out of 14 participants with TG >150 mg/dL, 5 (35.71%) participants were found to have prediabetes. Whereas out of 136 participants with TG ≤150 mg/dL, 21 (15.44%) participants were found to have prediabetes. The association between prediabetes and higher TG levels was not significant (p-value=0.056).

Triglycerides	Prediabetes		Total
	Yes	No	
>150 mg/dL	5 (35.71%)	9 (64.29%)	14 (100%)
≤150 mg/dL	21 (15.44%)	115 (84.56%)	136 (100%)
Total	26 (17.30%)	124 (82.70%)	150 (100%)

[Table/Fig-9]: Association between triglycerides of the participants and prediabetes.
Chi-square=3.64; p-value=0.056

[Table/Fig-10] shows that mean TC (204.88±47.99 and 152.46±29.35 mg/dL), mean LDL (94.77±30.71 and 69.57±17.88 mg/dL), mean VLDL (128.08±49.96 and 39.52±39.99 mg/dL) and mean TGs (48.65±18.45 and 28.18±9.47 mg/dL) were significantly high in prediabetes participants compared with healthy participants (p<0.05). Whereas difference between mean HDL levels (57.65±4.59 and 66.18±6.02 mg/dL) was not found to be significant (p-value=0.582).

Lipid profile (mg/dL)	Prediabetics		Healthy Participants		p-value
	Mean	Std. Dev.	Mean	Std. Dev.	
Total cholesterol	204.88	47.99	152.46	29.35	<0.001
HDL	57.65	4.59	66.18	6.02	0.582
LDL	94.77	30.71	69.57	17.88	0.019
VLDL	128.08	49.96	39.52	39.99	0.011
Triglycerides (TG)	48.65	18.45	28.18	9.47	<0.001

[Table/Fig-10]: Comparison of mean values of lipid profile between prediabetics and healthy participants.
Unpaired t test; p-value <0.05 considered significant

DISCUSSION

The present study is among the few studies of Indian population assessing the association between serum lipid profile and prediabetes in adult off-springs of known cases of type 2 diabetics. In the present study, mean age of the participants was 25.01±5.55 years ranging from 18 to 36 years. Whereas, in a study conducted by Pandey U et al, the mean age of male participants was 18.5±1.5 years and the

mean age of females was 17.9±1.8 years [10]. Similarly, in a study conducted on adults by Woldegebriel AG et al., the mean age of the study subjects was 39.3 years [13]. The majority of the respondents (71.75%) were in the age group of 24-44 years. Also, in the study by Bisht I et al., mean age of the participants was 45.89±9.35 years [14].

It was found that occurrence of prediabetes was maximum in age group 26 to 30 years (26.67%) followed by 17.64% in age group ≤20 years but association between age of the participants and occurrence of prediabetes was not found to be statistically significant (p-value=0.749). Considering the gender-wise distribution of the participants, out of 150 participants, 83 (55.3%) were females and 67 (44.7%) were males. In the study conducted by Ramya HS et al, with 389 adolescent participants, 45% were girls and 55% were boys [15]. Bisht I et al., in a study with 80 apparently healthy subjects, had more male participants 45 (56.25%) than female participants 35 (43.75%) [14]. Also, in a study conducted by Pandey U et al., out of 526 subjects, 277 (52.66%) were boys and 249 (47.34%) were girls [10].

It was found that occurrence of prediabetes was more in male participants (25.37%) as compared to female participants (10.84%). Association between gender of the participants and occurrence of prediabetes was found to be statistically significant (p-value=0.019). Comparable findings were seen in a study by Bisht I et al., with more prevalence of prediabetes in males, but occurrence of prediabetes was not significant (p-value=0.896) [14]. This was different from the results of Spurr S et al., study which showed no significant difference between men and women diagnosed with prediabetes [16].

In the present study, 26 (17.3%) participants were found to be pre-diabetic (fasting BLS 100 to 125 mg/dL). Whereas in the study conducted by Pandey U et al., prevalence of prediabetes among the study subjects was found to be 32.1% [10]. The difference between findings of these two studies may be due to the different criteria used for estimating glucose intolerance as present study measured fasting plasma glucose levels and the study by Pandey U et al., measured the plasma glucose levels two hours following Oral Glucose Tolerance Test (OGTT). Indian Council of Medical Research (ICMR)-INDIAB study, found the prevalence of diabetes to be 10.4% in Tamil Nadu, 8.4% in Maharashtra, 5.3% in Jharkhand and 13.6% in Chandigarh and the prevalence of prediabetes to be 8.3%, 12.8%, 8.1% and 14.6%, respectively [17]. The Delhi Urban Diabetes Survey (DUDS) done by Madhu SV et al, showed a prevalence of prediabetes as 21% using WHO criteria and 39.5% using American Diabetic Association (ADA) criteria [18].

The association between prediabetes and higher TC levels, lower HDL levels, higher LDL levels, higher VLDL levels was found to be statistically significant (p-value <0.05) and the association between prediabetes and higher TG levels was not significant (p-value=0.056). These findings are consistent with the study by Kansal S and Kamble TK which showed that prediabetes was significantly associated with higher TC levels, lower HDL levels, higher LDL levels, higher VLDL levels (p-value <0.05) [19].

In the present study, mean TC (204.88±47.99 and 152.46±29.35 mg/dL) was significantly high in prediabetic participants compared with healthy participants. Findings of present study are in accordance with a study by Bisht I et al., which showed that, mean cholesterol level was significantly higher among prediabetics when compared with healthy participants (199.65±51.96 and 176.75±39.17 mg/dL respectively) [14]. Same observations were seen in a study by Kansal S and Kamble TK where the mean value of total cholesterol for cases (184.75±46.02 mg/dL) was significantly more than controls (170.99±38.27 mg/dL) [19].

Mean LDL (94.77±30.71 of prediabetics and 69.57±17.88 mg/dL of non prediabetics), was found to be significantly high in prediabetic participants compared with healthy participants. The findings are in support with a study by Kansal S and Kamble TK where the mean LDL value for case (120.39±38.34 mg/dL) was significantly more than controls (99.84±29.57 mg/dL) [19]. Also, mean VLDL was significantly high in prediabetic participants (128.08±49.96) compared with healthy participants (39.52±39.99 mg/dL) in the present study. The findings were in agreement with the study by Kansal S and Kamble TK with mean value of VLDL for case (29.07±20.08 mg/dL) significantly more than controls (22.27±14.32 mg/dL) [19]. But, the difference between mean HDL levels between prediabetics (57.65±4.59) and healthy individuals (66.18±6.02 mg/dL) was found to be non significant in the present study. Although, the association between prediabetes and lower HDL levels, was found to be statistically significant (p-value <0.05)

In the present study, mean TG level was significantly higher in prediabetic participants (48.65±18.45) compared with healthy participants (28.18±9.47 mg/dL). Similar findings were reported in a study by Kansal S and Kamble TK with the value of triglyceride for cases (139.5±47.24 mg/dL) significantly higher than controls (106.81±61.97 mg/dL) [19]. Rahbar S also reported that prediabetics are having high triglyceride (TG) levels [20]. Also, Barzi F et al., Gaziano JM et al., and Boizel R et al., found that TG levels were significantly higher in participants with impaired fasting glucose than participants with normal fasting glucose [21-23].

In clinical practice, lipid abnormalities are monitored using parameters like Total Cholesterol, Triglycerides, High Density Lipoproteins, Low Density Lipoproteins, Very Low-Density Lipoproteins. Though cardiovascular complications related to lipid disorders are significant contributors to the costs of diabetes care, evidence-based recommendation for screening of lipid disorders is not available [24]. Many studies showed that higher rates of diabetes-related microvascular and macrovascular complications are a result of less diagnosis and delayed treatment of lipid disorders [25,26]. Treatment with lipid lowering drugs benefits T2DM patients more than non diabetic patients as shown by a systematic review and meta-analysis of Randomised Controlled Trials (RCTs) [27]. Therefore, it is recommended that early screening and correction of lipid disorders should be included in management of prediabetes and prevention of T2DM complications.

Limitation(s)

The nature of the study design (cross-sectional) was such that recording of parameters was done only on single occasion. The reported findings were of prediabetic patients; so, the results may not be generalised. Other information, like lifestyle variables were not considered.

CONCLUSION(S)

In the present study, prevalence of prediabetes was 17.3%. Prevalence of prediabetes was more in age group 26 to 30 years and in male participants. Total cholesterol, LDL, TG, VLDL were significantly raised whereas HDL was significantly lower in prediabetic individuals as compared to non prediabetic healthy subjects. Thus, dyslipidaemia puts prediabetic diagnosis and timely treatment can help to prevent/decrease serious complications of diabetes. So, young adults with family history of diabetes mellitus needs to be identified for regular screening of blood sugar and lipid profile as the onset of glucose intolerance can occur in the young adults. In future, the same study can be conducted in prospective way with a large cohort of prediabetic patients by grouping subjects depending on changes in lipid profiles over a length of considerable follow-up period instead of considering them as a single index.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Physiology, Government Medical College, Miraj, Maharashtra, India.
2. Assistant Professor, Department of Physiology, Government Medical College, Miraj, Maharashtra, India.
3. Professor, Department of Physiology, RCSM GMC, Kolhapur, Maharashtra, India.
4. Professor, Department of Physiology, Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Atish Bhaskar Pagar,
Prarambh, Bunglow Number 3, Bakul Bag, Near Sanglikar Mala, Miraj,
Sangli-416410, Maharashtra, India.
E-mail: abp123098@gmail.com

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