ORIGINAL RESEARCH PAPER

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EFFECT OF IRON DEFICIENCY ANEMIA ON HbA1c IN DIABETIC, PRE-DIABETIC AND NON-DIABETIC PATIENTS- IS THERE A DIFFERENCE??

KEY WORDS: Diabetic, glycated haemoglobin, iron deficiency, iron profile , pre diabetic, RBC indices

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ABSTRACT

Background and objectives: HbA1c is routinely used to follow up blood sugar levels and is a useful index of chronic hyperglycaemia. Besides blood sugar, several factors like hemoglobinopathies, nutritional deficiencies etc. affect HbA1c levels. Present study was conducted to study the effect of IDA on HbA1c levels in Indian diabetic, pre-diabetic and non-diabetic adults. **Materials & Methods:** This is a retrospective cross section study consisting of 1657 patients over a period of 3 months. The diabetic group, prediabetic and normal control groups comprised of 251 ,373 and 1023 patients respectively . **Results:** The mean HbA1c levels were significantly lower in IDA diabetic group as compared to non-IDA diabetic group. In the prediabetic group the HbA1c levels were higher in IDA group as compared to non-IDA group but the difference was not statistically significant. In the normal control group the mean HbA1c levels were significantly higher in IDA group as compared to non-IDA group. HbA1c did not show significant correlation with RBC indices or iron profile parameters. **Interpretation & Conclusion:** HbA1c levels are higher in IDA patients in pre diabetic and normal controls. On contrary HbA1c levels are lower when IDA coexists with diabetes. It is highly advisable to correct the IDA deficiency in all patients before diagnosing diabetes as well as correction of IDA once the diagnosis so that the HbA1c levels are actual depiction of glycaemic control.

INTRODUCTION:

HbA1c is routinely used to follow up blood sugar levels and is a useful index of chronic hyperglycaemia.

Several factors affect HbA1c levels besides blood sugar, including hereditary disorders, acute or chronic blood loss, haemolytic anaemia and nutritional deficiencies.

Nutritional deficiencies are highly prevalent worldwide and in India. Worldwide, IDA is most prevalent type of nutritional anaemia and contributes to 50% of the total anaemia. (1,2) In India, the prevalence of anaemia is 25 % in men aged 15- 65 years and 57 % in females aged 15-65 years. (3)

Various studies have demonstrated that HbA1c levels are increased in IDA while few other studies not found any significant correlation (4-7). Thus the results are conflicting; also only limited studies are available from India where the nutritional deficiencies are highly prevalent.

Hence the present study was conducted to analyze the effect of IDA on HbA1c levels in diabetic, pre-diabetic and non-diabetic adults.

MATERIALS AND METHOD:

This is a retrospective cross section study and all patients enrolling for blood tests over a period of 3 months were included in the study. The results of HbA1c, CBC and iron profile were analysed.

Groups were divided into normal, pre diabetic and non-diabetic on basis of history and HbA1c levels following ADA guidelines (8).

Anaemia was defined as Hb < 13 g/dl in males and <12 g/dl in females. All the three groups were further divided into IDA and non-IDA and males and females.

Inclusion Criteria:

All cases enrolled for routine blood examination in age group 18-65 years.

Exclusion Criteria:

Known cases of hemoglobinopathy, chronic liver disease, pregnancy and lactation, malignancies, trauma and anaemia caused by cases other than iron deficiency or any chronic /acute illness and patients not undergoing these tests.

Statistical Analysis:

Data was compiled in Microsoft excel and analysed using SPSS version 22.

RESULTS:

A total of 1657 patients were included in the study and divided into three groups based on HbA1c level. The diabetic group comprised of 251 patients, the pre diabetic group comprised of 373 patients and 1023 patients formed the normal control group. The groups were further divided into IDA and non-IDA subgroups. Table 1 shows the demographic details.

Table 1: Demographic Details Of Study Population

	Normal group		Pre Diabetic group		Diabetic group	
	IDA	Non IDA	IDA	Non IDA	IDA	Non IDA
Total number	392	631	109	264	71	180
Male	62	373	28	163	34	113
Female	330	258	81	101	37	67
Mean Age (yrs)	36.12 ± 10.73	33.77 ± 9.95	47.63 ± 14.38	46.05 ± 13.14	56.23 ± 13.19	51.15 ± 12.46
Male Mean age (years)	38.61 ± 14.3	36.42 ± 9.33	52.11 ± 15.88	43.87 ± 11.64	58.44 ± 13.33	48.03 ± 11.63
Female Mean Age (years)	35.65 ± 9.88	34.84 ± 10.74	46.09 ± 13.59	49.57 ± 14.64	54.19 ± 12.91	56.42 ± 12.11

Diabetic Group:

A total of 251 patients were included in the diabetic group. 71 patients fomed IDA subgroup and 180 were in non-IDA subgroup. Table 2 shows the mean and p values of the various parameters analysed.

Table 2: Mean Values Of Various Parameters In Diabetic Groups

Parameters	IDA diabetic group	Non IDA diabetic group	p value	IDA diabetic male subgroup	Non-IDA diabetic male subgroup	p value	IDA diabetic female subgroup	Non-IDA diabetic female subgroup	p value

HbA1c (%)	7.91 ± 1.81	8.46 ± 1.77	0.029	8.38 ± 2.26	8.49± 1.72	0.756	7.48± 1.15	8.39± 1.86	0.007
Average blood glucose (mg/dl)	180.29± 52.02	195.96± 50.84	0.029	193.79± 64.74	197.03± 49.5	0.756	167.88± 33.03	194.17 ± 53.35	0.007
Hb(g/dl)	11.02± 1.38	13.9 ± 1.13	<0.001	11.51 ± 1.49	14.45 ± 0.98	<0.001	10.57 ± 1.1	12.98± 0.68	<0.001
MCH (pg)	24.39± 4.35	27.52± 2.14	<0.001	25.67 ± 5.04	27.91 ± 2.08	<0.001	23.21 ± 3.24	26.86± 2.11	<0.001
MCV (fl)	77.81± 11.16	84.89± 5.59	<0.001	80.52 ± 13.02	85.71 ± 5.04	<0.001	75.32 ± 8.32	83.6± 5.67	<0.001
MCHC (g/dl)	31.21± 1.26	32.4 ± 0.93	<0.001	31.73 ± 1.17	32.55 ± 0.97	<0.001	30.73 ± 1.16	32.15± 0.81	<0.001
Iron (µg/dl)	51.45± 25.9	95.66± 28.26	<0.001	56.73 ± 26.37	96.62 ± 27.84	<0.001	46.6± 24.83	98.42± 29.12	0.047
TIBC (µg/dl)	351.14± 72.16	303.42± 61.68	<0.001	361.55± 84.64	303.93± 63.56	<0.001	353.9 ± 59.34	302.58 ± 58.85	<0.001
UIBC (µg/dl)	305.69± 67.61	202.36± 47.83	<0.001	304.82± 79.79	207.33± 52.98	<0.001	306.48± 55.24	204.29 ± 37.88	<0.001
Transferrin (%)	14.57± 6.46	31.86± 5.7	<0.001	15.98 ± 6.86	31.86 ± 6	<0.001	13.28 ± 6.48	32.15± 5.19	<0.001

The mean CBC indices were significantly lower in IDA group as compared to non-IDA group overall as well in males and females. (Table 2). Though HbA1c values were lower in IDA group as compared to non IDA group, the difference was statistically significant only for females. The mean Iron, and transferrin saturation of IDA and non-IDA were significantly lower and UIBC and TIBC were significantly higher in IDA group as compared to non-IDA group, overall as well in males and females. (Table 2).

HbA1c values did not significantly correlate with RBC indices or iron profile.

Pre-diabetic Group:

A total of 373 patients were included in the pre-diabetic group, comprising of 109 IDA and 264 non-IDA patients. Table 3 shows the mean and p values of the various parameters .

Table 3: Mean Values Of Various Parameters In Pre-Diabetic Groups

Parameters	IDA pre-diabetic group	Non IDA pre-diabetic group	p value	IDA pre-diabetic male subgroup	Non-IDA pre-diabetic male subgroup	p value	IDA pre-diabetic female subgroup	Non-IDA pre-diabetic female subgroup	p value
HbA1c (%)	6.05 ± 0.21	6.02 ± 0.21	0.294	6.08± 0.22	6.02± 0.21	0.217	6.04± 0.21	6.03 ± 0.21	0.66
Average blood glucose(mg/dl)	126.09± 6.16	126.18± 5.9	0.294	127.65± 6.23	126.15 ± 5.91	0.217	126.63 ± 6.15	126.24± 5.9	0.66
Hb (g/dl)	11.08± 1.27	13.09± 1.15	<0.001	11.95 ± 1.06	14.59 ± 0.8	<0.001	10.78± 1.21	12.78 ± 0.67	<0.001
MCH (pg)	24.43± 3.52	27.74± 2.07	<0.001	25.51 ± 3.16	28.1± 2.14	<0.001	24.06± 3.58	27.15 ± 1.83	<0.001
MCV (fl)	77.98± 9.11	85.96± 5.62	<0.001	80.7± 8.24	86.59 ± 5.81	<0.001	77.04± 9.26	84.96 ± 5.17	<0.001
MCHC (g/dl)	31.23± 1.22	32.25± 0.84	<0.001	31.56 ± 1.1	32.43 ± 0.85	<0.001	31.12± 1.25	31.96 ± 0.75	<0.001
Iron (µg/dl)	56.92± 29.05	101.23± 27.09	<0.001	82.43 ± 33.18	97.39 ± 28.34	0.013	48.1± 21.48	107.42± 26.13	<0.001
TIBC (µg/dl)	393.75± 44.59	307.45± 55.85	<0.001	407.69± 50.42	303.13 ± 57.14	<0.001	384.94 ± 41.65	314.43± 53.25	<0.001
UIBC (µg/dl)	336.83± 29.49	206.3± 37.45	<0.001	325.26 57.82 ±	205.79 ± 39.58	<0.001	340.84 ± 45.7	207.13± 33.9	<0.001
Transferrin (%)	14.53± 7.34	32.63± 5.29	<0.001	20.44 ± 8.29	31.87 ± 5.62	<0.001	12.49± 5.75	33.86 ± 4.46	<0.001

The mean Hb, MCV, MCH and MCHC of IDA and non-IDA were significantly lower in IDA group as compared to non-IDA group overall as well as in male and female subgroups. The HbA1c levels were higher in IDA group as compared to non-IDA group overall and in males and females, but the difference was not statistically significant. The iron profile paramewters showed a significant difference in both males and females. Though HbA1c was higher in IDA group as comopared to non IDA group, the difference was not statistically significance.

HbA1c did not significantly correlate with RBC indices or iron profile in this group.

Normal Control Group:

A total of 1023 patients were included in the normal control group, including 392 IDA and 631 non IDA patients. Table 4 shows the mean and p values of the various parameters in this group.

Table 4: Mean Values Of Various Parameters In Normal Control Groups

Parameters	IDA group	Non IDA group	p value	IDA male subgroup	Non-IDA male subgroup	p value	IDA female subgroup	Non-IDA female subgroup	p value
HbA1c (%)	5.35± 0.26	5.34 ± 0.26	<0.001	5.38± 0.23	5.36 ± 0.26	0.676	5.35 ± 0.27	5.3± 0.27	0.041

Average blood glucose(mg/dl)	106.97± 7.52	106.49± 7.55	<0.001	107.57± 6.67	107.15± 7.38	0.676	106.85± 7.68	105.55 ± 7.71	0.041
Hb (g/dl)	10.87± 1.44	14.08± 1.26	<0.001	11.95 ± 1.48	14.85± 0.92	<0.001	10.67± 1.34	12.96± 0.74	<0.001
MCH (pg)	24.56± 4.2	28.44± 2.22	<0.001	26.93 ± 5.84	28.76± 2.36	<0.001	24.12± 3.66	27.97± 1.9	<0.001
MCV (fl)	78.5 ± 10.76	87.04± 5.98	<0.001	84.02 ± 15.59	87.53± 6.45	0.002	77.46± 9.26	86.34± 5.17	<0.001
MCHC (g/dl)	31.14± 1.51	32.66± 0.91	<0.001	31.9± 1.4	32.86± 0.93	<0.001	31± 1.48	32.38± 0.81	<0.001
Iron (µg/dl)	82.12± 38.36	95.5 ± 28.34	<0.001	89.32 ± 45.45	94± 28.29	0.257	80.75± 38.8	97.68± 28.32	<0.001
TIBC (µg/dl)	323.4± 68.54	300.96± 62.07	<0.001	320.74± 76.2	300.38± 65.1	0.027	323.48± 67.12	301.78 ± 57.74	<0.001
UIBC (µg/dl)	240.93± 81.42	205.52± 47.57	<0.001	231.31± 87.44	206.45± 52.84	0.002	242.73± 80.25	204.19 ± 38.79	<0.001
Transferrin (%)	26.57± 11.4	31.62± 5.73	<0.001	29.12 ± 10.43	31.31± 5.93	0.019	26.09± 11.6	32.06± 5.41	<0.001

The mean Hb, MCV, MCH and MCHC were significantly lower in IDA group as compared to non-IDA group. The mean HbA1c levels were significantly higher in IDA group as compared to non-IDA group, similar to as obtained in pre-diabetic group. Female subgroups of IDA and non-IDA groups also showed similar results. In the male subgroup, Hb, MCV, MCH and MCHC were significantly lower in IDA group as compared to non-IDA group. Though HbA1c levels were also lower in IDA group in male category, the difference was not significant.

The Iron profile showed a significant difference in the IDA and non IDA groups. For serum iron though the values were lower in IDA group as compared to non-IDA group for both males a females , the difference in Iron values was only significant for females.

HbA1c did not significantly correlate with RBC indices or iron profile in normal control group as well.

DISCUSSION:

In the diabetic study population, the mean HbA1c levels were 7.91 ± 1.81 in IDA group and 8.46 ± 1.77 in non-IDA group. The difference was statistically significant ($p < 0.05$). Similar results were obtained by Solomon et al in year 2019 in their study on 174 diabetic patients. The reason for low HbA1c in IDA patient as compared to non-IDA patients is the degree of anaemia in the study population (4). Several authors have studied effect of IDA on HbA1c in non-diabetic patients. They all concluded that HbA1c concentration tends to be lower in the presence of iron deficiency anaemia. (9-13).

The HbA1c values were significantly lower in diabetic female IDA pt. as compared to non-IDA patients. In the male category though the HbA1c values were lower in diabetic IDA subgroup the difference was not statistically significant. The difference between different results in male and female category could be due to the reason that females are physiologically more susceptible to development of anaemia, most commonly nutritional anaemia due to different dietary habits, hormones and loss of blood during menstrual cycles.

A significant difference was obtained in Hb and RBC indices and iron profile in IDA and non-IDA groups in both males and females subgroup. However the correlation of HbA1c with these parameters was not statistically significant. These findings are similar to as obtained by several other authors (4, 13, 14).

In the pre-diabetic study population, the mean HbA1c levels were 6.05 ± 0.21 in IDA group and 6.02 ± 0.21 in non-IDA group. Though HbA1c was higher in pre-diabetic IDA pt. the difference was not statistically significant. Also HbA1c was higher in both pre-diabetic amle and female IDA pt. as compared to non IDA patents, but the difference was not statistically significant. Madhu SV et al in their study on 62 IDA patients and 60 normal controls also observed higher HbA1c levels in IDA patients as compared to non-IDA patients. They hypothesized that IDA leads to false high estimation of HbA1c levels and hence should not be used as sole criteria for diagnosis of diabetes. They observed a strong negative correlation of HbA1c with iron profile (15). The current study shows a significant difference in Hb , RBC indices and iron profile in IDA and non-IDA male and female groups with a significant correlation with. The difference in findings could be due to large sample size in present study.

In the normal control study population, the mean HbA1c levels were 5.35 ± 0.6 in IDA group and 5.34 ± 0.26 in non-IDA group and the difference was statistically significant. The mean HbA1c values were also significantly higher in normal male and female IDA group as compared to non IDA subgroup. These findings are similar to those obtained by several authors. Ford et al., in 2011, Silva et al., in 2015, Shekhae et al., in 2014 and Chhabra et al., in 2015 also obtained higher HbA1c level in IDA patients(12, 16-18). On contrary Sinha et al., in 2012, Cavagnolli et a., in 2015 and Kalasker et al., in 2014, obtained HBA1c as lower in IDA group (9-11). In present study a significant difference was obtained in Hb and RBC indices and iron profile in IDA and non-IDA groups as well as between males and females subgroup. However the correlation of HbA1c with theses parameters was not statistically significant.

Various propositions have been made to explain the increase in glycated haemoglobin level in IDA patients. Coban E et al proposed that in IDA there is alteration of quaternary structure of haemoglobin and hence glycation of beta chain occurs more readily (19). El Agouza et al proposed that the increase in glycated Hb levels in non-diabetic anaemic patients occurs due to decrease in Hb levels of these patients (20).

The current study is the largest study on Indian population studying the effect of IDA on HbA1c in diabetic, pre-diabetic and normal controls. Having a larger size of study population, we suggest that IDA causes an elevation in HbA1c levels in pre-diabetic and normal controls. But in the diabetic population, the levels of HbA1c are lower in IDA group as compared to non-IDA group. This could be due to effect of several confounding factors. These confounding factors tend to lower Hb levels more, and include effect of pro inflammatory cytokines and micro and macrovascular complications of diabetes like diabetic nephropathy (21). This study becomes highly relevant in Indian scenario as there is a very high prevalence of IDA in Indian population. HbA1c besides being effected by blood glucose levels, also gets effected by level of haemoglobin, as well as nutritional deficiencies. As there is a significant difference in HbA1c levels in IDA and non-IDA groups for diabetic, pre-diabetic as well as normal control, we highly recommend to correct the IDA deficiency in all patients before diagnosing diabetes as

well as correction of IDA once the diagnosis is made as HbA1c levels are used to monitor glycaemic control in these patients.

CONCLUSION:

The results in current study show that HbA1c levels are higher in IDA patients in pre diabetic and normal controls. On contrary HbA1c levels are lower when IDA coexists with diabetes. As HbA1c is used to diagnose as well as monitor diabetes we highly recommend correction of iron deficiency before clinical diagnosis as well as when seen during monitoring, so as to correctly depict the glycaemic control.

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