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How dietary protein and vitamin C affect anemia and not iron alone: early pregnancy data from a North Indian Hospital.

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Objective: fetal development starts during the first trimester of pregnancy and the mother's diet during this period is crucial. The aim of this study is to investigate possible associations of major dietary components of protein fat energy and vitamin C iron from diet and level of hemoglobin (Hb) as few studies have investigated the role of dietary sources of iron in relation to anemia (Hb<11gm%).

Design: two hundred twenty-six primi para women of mean age 23 years, age of menarche 14 years, belonging to middle-income group, graduate by education attending tertiary care center for the antenatal care of their first pregnancy in a metro city of Delhi provided their diet information. They completed the background information, fasting blood samples for testing using standard hospital machines, and diet intake as per 24-hour recall methods for diet content, portion size, and frequency for the whole day for 3 days to calculate average fat, protein, energy iron, and vitamin C details calculated using validated tools.

Results: First trimester BMI and biochemical parameters were the same in the anemic (Hb<10.9gm% and nonanemic group (Hb>11gm%). Consumption for major food components less than RDA was 46.8%-energy, 16.4%-protein, 1.2%-Fat, 8.4% iron, 16.8% vitamin C. Among anemic <RDA consumption was higher- for fat1.0%, protein19.6%, energy-52.9%, iron10.8% and vitamin C13.7%. However not different statistically significant. Extrapolation for trend analysis showed rising hemoglobin with rising vitamin C and no trend or U shape curve with energy protein. High Fat intake was found to trend for the lower side of Hemoglobin.

Conclusion: Though a bigger sample is required for conclusive outcomes the study data/trend analysis indicates that a diet without any supplements becomes a rich source of iron/rising Hb if it is high in vitamin C content and low in fat.

Body Text:

"How dietary protein and vitamin C affect anemia and not iron alone: early pregnancy data from a North Indian Hospital".

Background:

Iron deficiency anemia control is an unresolved issue in India[1]. Various researches and efforts are ongoing to eradicate anemia. In India since 1971 Anemia control program (now refined as "Anemia Mukt Bharat"[2]) have done many strategic changes to improve the situation which is continuously unaltered[3] as the prevalence of anemia during pregnancy has not changed over the years[4].Current policy is focusing on increasing consumption of number of iron tablets by pregnant women[5]. Many strategies are implemented to improve availability of Iron supplements and to enhance consumption of iron tablets at least for 90 days during pregnancy. Changing the formulations from ferrous sulphate to ferric carboxy maltose/ ferric sucrose versus ferric ammonium sulphate etc are also being tried[6]. Despite many efforts done to improve iron status the NFHS surveys done over the years have shown increasing prevalence of anemic women 49.7% in NFHS-2, 58.7% in NFHS-3 50.3% in NFHS-4 (2015-16), and 56.5% in NFHS 5 done in 2020-21[7]. Six strategies under anemia Mukt bharat are striving for situation change however among all these strategies-deficient wholesome diet is found significantly associated with microcytic anemia. Among 305 pregnant mothers who were supplemented with iron during pregnancy[8] in urban hospital to find how much iron alone and how whole diet affect anemia. The study showed that complete Food/Whole diet plays major role with microcytic anemia (x^2 = 26.8) and dietary deficiency had a sensitivity of 71%, specificity of 79%, and negative predictive value of 97% (222 of 229) when compared with iron supplementation alone[6]. Women living in food insecurity zone were found 1.6 times more likely to suffer from anemia compared to their food secure counterparts[9]. Seeing this much importance of food in raising hemoglobin levels and combating anemia we conducted a secondary analysis on available ICMR study diet data[10] where about half of the study participants diet intake information (n=250) was available from enrolled 500 study participants. Secondary data analysis is done to see how dietary protein, caloric intake, fat, vitamin C and iron is associated with change in the level of hemoglobin.

Role of protein and vitamin C is emphasized in various studies to improve bioavailability of dietary iron[11] and reduced anemia. In view of various researches pointing out on role of whole diet rather only focus on increasing consumption of tablet of iron supplementation[12] this secondary analysis of available data of pregnant women in their primi pregnancy first trimester was done to see if hemoglobin is affected by dietary iron or role of protein, vitamin C in middle income group metro city pregnant women attending tertiary care center for normal pregnancy management.

Methods:

This analysis of diet data from the study where subgroup of half of pregnant women's diet details were available from collected data under the study done at tertiary care centers of north India during 2011-15. The study was to see effect of daily iron versus weekly iron on the level of hemoglobin oxidative stress and birth outcome. The baseline detailed diet data using 24 hours dietary recall method[13] was collected on randomly selected half of study participants at the time of enrollment in the study during first trimester of their pregnancy which was not analyzed as primary objective. To elaborate diet intake the participants were shown the utensils of different sizes to record the portion size. Total amount of cooked food for whole family and part consumed by the pregnant women was recorded using validated Performa for total Diet assessments described by Gemma Salvador Castell et al[13]. Detailed daily consumption of food items and calculation of calories –energy, protein, fat, iron and vitamin C content of portion size in women's diet was done using standard methods by Castell et al[13]. Morning blood samples were collected to measure hemoglobin and other study parameters. Though the diet data was not analyzed that time as it was not primary objective, however to see effect of whole diet on hemoglobin levels diet data analysis is being submitted now in view of some important trend visible in this data after analysis which are of policy importance and required to be published for effective strategies for anemia mukt bharat. Primary objective data analysis was published in the paper by Harishankar et al[14] where main objective of daily versus weekly iron showed equal effect of daily versus weekly iron on birth outcome. Women under the study were grouped in anemic versus non-anemic on the basis of their hemoglobin levels[15] in first trimester of their pregnancy. Women with hemoglobin between 8 to 10.9 gm% labeled as anemic and hemoglobin ≥ 11 gm% are considered as non-anemic group.

Enrollment inclusion criteria involved women from 20-30 years, Hb% ≥ 8 gm%, women in first trimester (9 to 12 week), primigravida with no associated complication, middle income literate group (Kuppuswamy score 11-25) while excluded those having chronic ailments, already on any iron preparation in last three months. After informed written consent from participants and ethical committee approvals, the total data of whole diet was collected from 250 pregnant women at baseline in first trimester of pregnancy. Among them 226 had information on all parameters. 124 of them were having Hemoglobin levels above 11gm% (nonenemic) and 102 were having Hb below 10.9gm% (anemic). Filling of pre-coded proforma (CRF), diet recording, counseling, deworming was done by giving 400mg tablet of Albendazole at baseline. Appropriate statistical tests for statistical significance and correlation were applied using SPSS version 19 to find if there is any difference of various nutrients and dietary iron vitamin C on hemoglobin levels and trend of changing dietary factors with changing levels of hemoglobin. Recommended dietary allowances are analyzed using standards for RDA[16]. 1900-2800 K cal RDA for Energy, 47-68 (58) gm/day for protein, 20-30 gm / day for fat, 11-13 mg (21) per day for iron, 40-55mg/day for vitamin C has been taken as cutoff for analysis of food intake as per RDA[17].

Results:

Out of 250 women, data of 24 participants is excluded for incomplete information for some of the parameters under purview of this analysis. Hence only available 226 participant's complete information of diet and hemoglobin at first trimester of pregnancy is analyzed. The baseline physiological parameters of n=124 non anemic (Hb>11gm%) n=102 anemic (Hb 8 to 10.9gm%) are presented in Table1.

Table 1: Baseline (First Trimester) characteristics of physical parameters of blood tests and hemogram of study participants

Baseline vitals (first trimester)		S.Na (mEq/L)	S. K (mEq/L)	S. Ilbumin (gm/dl)	S. Globulin(gm/dl)	S. totalProtein(g/dl)	S.Creatine (mg/dl)	S.Alkaline Phosphatase (U/L)	S.Bilirubin (mg/dl)	Serum Cholesterol (mmol/L)	Serum Calcium
Non-anemic Hb >11gm%	Mean± SD*	139.8± 16.0	4.1±0 0.4	4.3±0 0.4	2.8±0. 4	7.2±0 0.4	0.5±0 0.1	133.4±64 .4	0.4± 0.2	170.1± 30.7	9.2±0. 5
Anemic Hb 8-10.9gm%	Mean± SD*	139.1± 3.8	4.1±0 0.4	4.1±0 0.5	2.9±0. 51	7.1±0 0.5	.5±0.3	130.0±48 .1	0.5±0 0.1	173.2± 30.8	9.0±0. 5

*not significant tly different statistically p value<0.05

Table 2: Physiological features of study participants

	Age at menarche (yrs.) Mean ±SD	Height (cm.) Mean ±SD	Weight (Kg) Mean ±SD	BMI Kg/M^2 Mean ±SD
Anemic Hb<10.9gm%	13.80±1.29	156.27±6.78	51.66±8.95	21.28±3.49
Non anemic-NA Hb>11gm%	14.14±2.01	156.30±7.78	53.67±14.39	21.41±4.05

Average age of menarche in both the groups was 14 years of age and age of marriage was 23 years.

Table 3 A: Overall Recommended Dietary Allowances (RDA) among all study participants n=250 without exclusion)

		N	%
Energy intake from diet	Below RDA	117	46.8
	Above RDA	133	53.2
Protein intake from diet	Below RDA	41	16.4
	Above RDA	209	83.6
Fat intake from diet	Below RDA	3	1.2
	Above RDA	247	98.8
Iron intake from diet	Below RDA	21	8.4
	Above RDA	229	91.6
Vitamin-C intake from diet	Below RDA	42	16.8
	Above RDA	208	83.2
	Total	250	100.0

Table 4A is showing major deficit of RDA for over all energy intake, protein,fat. The levels of iron and vitamin C intake level is sufficiently high among participants -highest RDA for fat intake.

Table 3B : RDA as per hemoglobin group of anemia and non anemia among n226 participants (24 participants data is not included due to incomplete information of all variables)

Diet content	RDA	Hb≥11m%	Hb 8-10.9gm%	Total
Energy intake from diet-N,%	Below RDA	57, 46.0%	54,52.9%	111, 49.1%

	Above RDA	67, 54.0%	48,47.1%	115, 50.9%
Protein intake from diet,N,%	Below RDA	18, 14.5%	20,19.6%	38,16.8%
	Above RDA	106,85.5%	82,80.4%	188,83.2%
Fat intake from diet N,%	Below RDA	1, 0.8%	1, 1.0%	2,0.9%
	Above RDA	123, 99.2%	101, 99.0%	224, 99.1%
Iron intake from diet-N,%	Below RDA	9, 7.3%	11, 10.8%	20, 8.8%
	Above RDA	115, 92.7%	91, 89.2%	206,91.2%
Vitaminc intake from die-N,%t	Below RDA	22, 17.7%	14, 13.7%	36, 15.9%
	Above RDA	102, 82.3%	88, 86.3%	190, 84.1%
Total, within Hb groups-N,%		124, 100.0%	102, 100.0%	226, 100.0%

Levene's Test for Equality of Variances applied (Independent Samples Test) when Equal variances assumed in all variables P value <0.05 is considered significant. Two sided t-test for Equality of Means showed no significant difference of diet intake

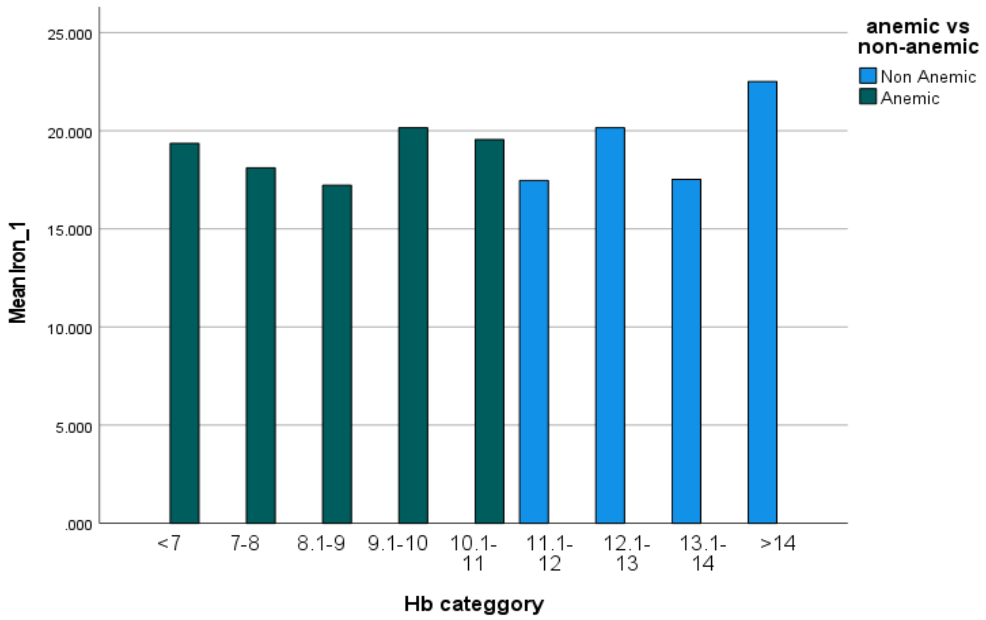
Table 4: Diet intake of 226 participants non anemic-NA (Hb>11gm%, n=124), and anemic (Hb<10.9gm%, n=102) calculated from ingredients consumed

Diet variable	Hb Group	Mean	SD	SE Mean	95% CI	Co relation with Hb and diet intake	P value
Energy (Kcal)	NA	1935.94	463.84	41.65	(87.9-168.6)	-.048	.49
	Anemic	1895.63	513.85	50.87			
Protein (gm)	NA	60.78	16.01	1.43	(-4.1-4.7)	-.028	.36
	Anemic	60.45	18.01	1.78			
Fat(gm)	NA	52.70	17.41	1.56	(-3.5-5.4)	-.030	.76
	Anemic	51.76	16.66	1.64			
Iron(mg)	NA	19.24	5.89	.52	(-1.2-2.0)	-.055	.09
	Anemic	18.83	6.77	.67			
Vitamin C (mg)	NA	98.48	75.57	6.78	(-31.7-8.9)	.075	.58

	Anemic	109.85	79.31	7.85			

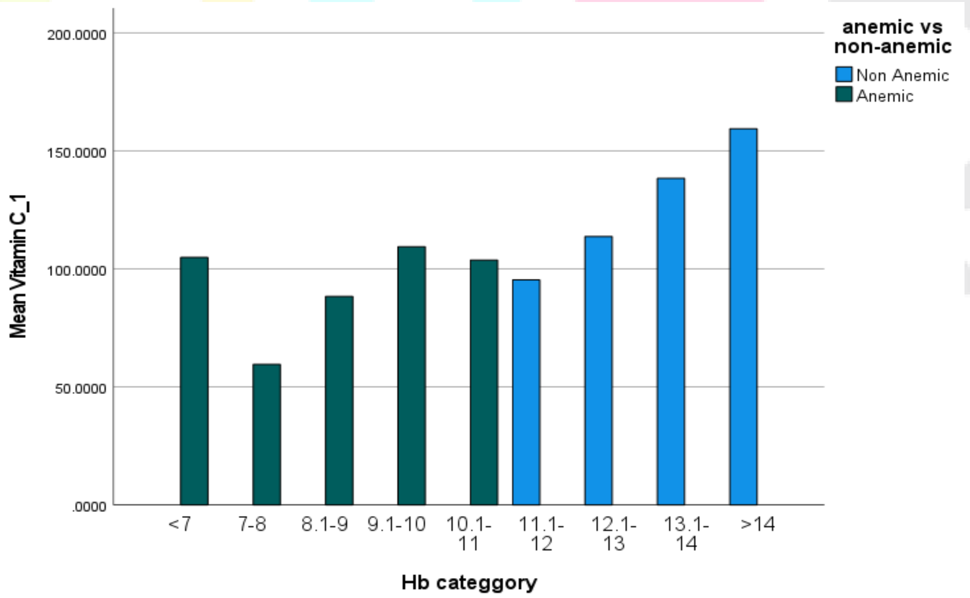
Table 3 is showing mean intake of food ingredients. Vitamin C is higher in non anemic group however no statistically significant difference of variables of nutrition shown affecting Hb level.

Graph 1



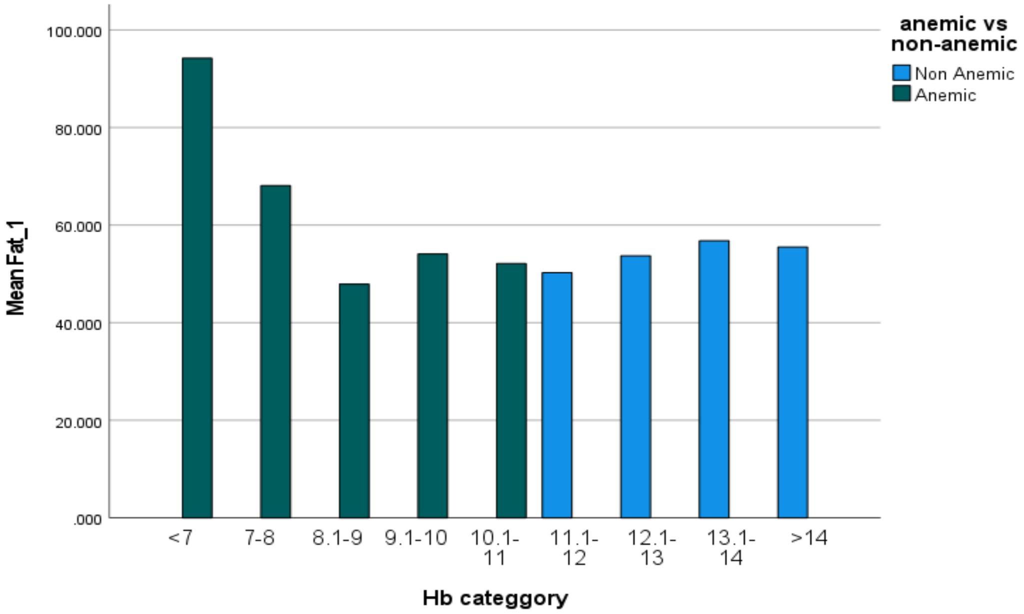
Graph-1 is showing gradual changing pattern of dietary iron and hemoglobin. With increasing hemoglobin levels iron consumption is not increasing.

Graph 2



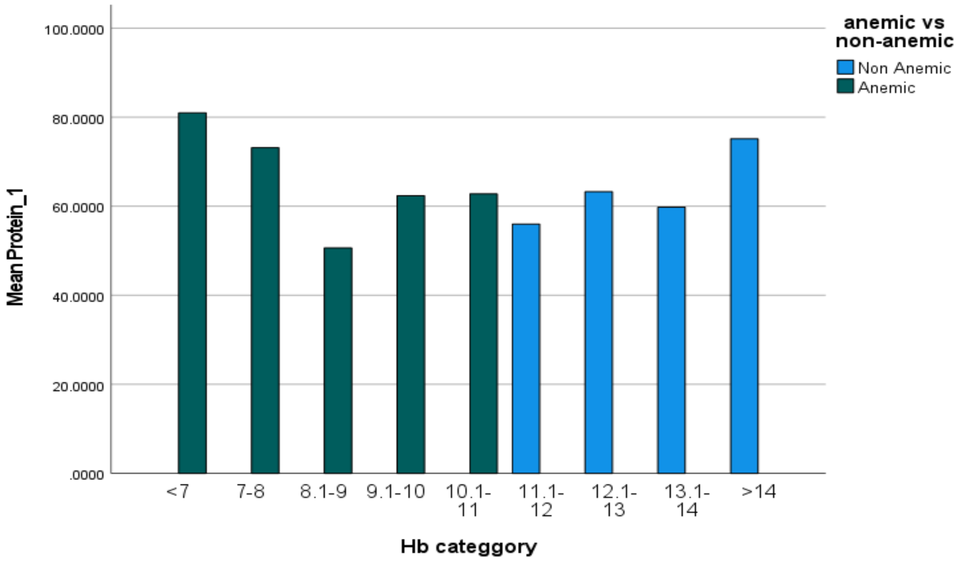
Graph 2 is showing increasing hemoglobin with rising intake of vitamin C in diet .

Graph 3



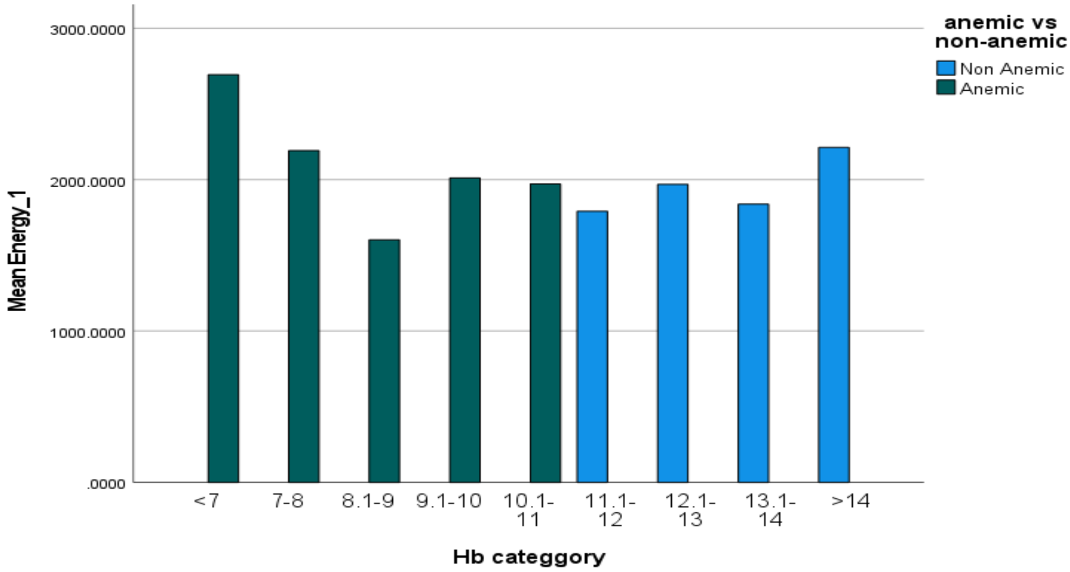
Graph 3 is showing trend towards decreasing/lowering levels of hemoglobin with rising intake of fat

Graph 4



Graph 4 is showing "U" shape pattern of protein intake where some sort of protein intake are causing low hemoglobin while other types are more with higher ranges of hemoglobin. The difference was checked and it was found that non vegetarian sources of protein were higher among those with low hemoglobin ranges.

Graph 5



Graph 5 is also showing pattern of "U" shape where despite increased energy hemoglobin levels are lower side. It is found that high energy source from fat is more among those wih low hemoglobins. More fats may be detrimental for hemoglobin levels. However type of consumed fat information is not obtained in detail in this data.

Discussions

There is functional significance of the covariance between Protein Energy Malnutrition and Iron Deficiency Anemia. Hemoglobin level of mother during pregnancy affect child's anemic status[17]. A review showed 56% women when found non compliant to iron intake were seen getting better with diet improvements[18]. World over, the majority of pregnant/ women have a dietary iron intake, below the recommended intake which is reported to contribute to a low iron status in pregnant women; however when analyzed for other nutrients, their diet protein was found affecting iron absorption in the body[19]. Like Tanzania study showed improving bio availability of dietary iron to improve anemia among women[20], low income countries like India- it is recommended to improve bio-availability from dietary resources[21]. also increasing food content and diversity have been proved successful in combating anemia[22]. The present data analysis is showing effect of dietary Vitamin C on hemoglobin level in positive trend. While dietary iron content is not much affecting Hb but rising level of vitamin C showing rising trend of Hb. Dietary protein, fat, and energy also found affecting hemoglobin levels as rising trend of hemoglobin is visible with rising intake of certain protein, energy. Increasing fat intake was found with lowered hemoglobin, however statistically significant outcome needs bigger sample size for conclusive outcome. Despite limitations, this data is good indicator for setting priority on improving whole diet of women rather only putting major resources in increasing number of iron tablet consumption. The strategy of increasing number of iron tablet consumption has not yield situation change as is evident from serial NFHS data and this secondary data analysis from primi pregnant women with no previous history of supplementation, only on regular diet shows that despite similar socio-economical status, age group, BMI, parity status and other similar conditions of living in same metro city/ locatility- their certain dietary contents like vitamin C could alter their hemoglobin levels. Whole diet in terms of energy, protein was showing trends towards rising hemoglobin while iron content was similar in both low and high hemoglobin ranges in diet and not found altered with hemoglobin levels. As evident from our data and information from other low income countries the recommended dietary allowances[23,24] are not consumed by majority population in average families of middle income group, yet vitamin C could make shift of hemoglobin towards more normal ranges.

Socio-demographic factors in Peru has shown the correlation to iron deficiency (Hb < 110 g/L) and no significant difference was there with different intakes of various nutrients including total iron and heme iron[25]. Role of vitamin C content and role in anemia is widely published and acknowledged[26] so is the finding of our study. Role of whole diet needs further attention in view of our findings and similar observations from published reports where use of diet information is found useful in getting anemic persons identified[27]. Diet is considered the major determinant of anemia in the London based study[28]. Whole Diet intake was found directly related to iron status in Australian study[29].

There is need to look in to fat amount in diet as high fat is observed with low hemoglobin in our data and similarly documented by others like Thomus et al[30]. There is a need to review policy of major focus on increasing iron consumption as it is showing little correlation as compared to high-fat and vitamin C. Caution required as iron overload is associated with insulin resistance, modified hepatic lipid and iron metabolism and increased mitochondrial dysfunction and oxidative stress[31]. Iron dysregulation is also reported with increased fat intake[32] hence further research on type and quantity of fats, its role in iron deficiency anemia is required. There is need to include multi-pronged approach of diversified diet, its content, bio-availability of content, processing, cooking methods and pots used in cooking, enhancers of iron absorption in food[33,34].

Limitations: Data is analyzed retrospectively which was taken back during 2013-2015 and need re validation in current scenario. In addition to the low power of the present study this data is a single time point data about hemoglobin and dietary intake collected using recall method hence inherited recall bias is there however it reflects per-pregnancy diet as well as diet and hemoglobin status in early pregnancy when no intervention is given. Another limitation is that we measured deficiencies of micro nutrients through dietary intake only. The strengths of this study include the large sample size and the assessment of pregnant female by well-trained teams within 6 weeks of pregnancy.

Indian data published in the year 2006 showed anemia and the role of diet[35]. Contrary to popular belief high energy in the form of fat intake was associated with low hemoglobin. In UK based study high BMI was found associated with low hemoglobin[36]. Role of protein fat and total energy in anemia has come up in our analysis, also has been highlighted in study done in China[37]. Very limited research trials are available on this topic that warrants a careful interpretation of results inferred and a considerable need of larger population-based studies and randomized controlled trials for better outcomes[38] and bring out suggested improvements[39]

Conclusions:

There is role of multip le nutrient and wholesome diet on iron status, hemoglobin and anemia status of pregnant women. It was found that there is need to do multi-pronged- multifaceted interventions for complete comprehensive individualized converge of many facets of anemia problem. Despite equal iron iron content in diet- the primi para women with higher hemoglobin were having higher protein and vitamin C intake. Hence wholesome diet is recommended to address iron deficiency anemia during pregnancy as focusing on increasing number of iron tablets may have limited role.

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