Low cholesterol and lowest deficiency of vitamin D & vitamin B12 in Indian old population in comparison to other age groups

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Abstract:
Background: None of the studies have evaluated the age and sex wise concentrations and prevalence of deficiency of vitamin D, vitamin B12, calcium, iron and cholesterol in Indian population. Therefore, the present study compared the prevalence of deficiency of these components in various age groups of Indian population.
Methodology: The serum concentrations for vitamin D, iron, vitamin B12, cholesterol and calcium were determined by an automated analyzer (CX 9; Beckman, Brea, CA) with the use of commercial kits (Beckman coulter, CA).
Results: Vitamin D and vitamin B12 deficiency was significantly higher in young population in comparison to older age group ($p<0.05$). Older age groups were found to have low cholesterol levels, high vitamin D and vitamin B12 levels when compared with other age groups. Female infants have lower prevalence of vitamin D (76% vs 62%; $p=0.03$) deficiency and female adults higher vitamin D concentration (Males: 23.66±14; Females 26.46±15; $p<0.0001$) when compared with age matched males. Vitamin B12 level was significantly higher in female teenagers, female adults and male infants. 71% of showed significantly high prevalence of iron deficiency (Female old: 50%, $p=0.0003$). Similarly old males (71% vs 50%; $p=0.0003$) and female infants (100% vs 78%; $p=0.0001$) showed significantly high prevalence of iron deficiency when compared with age matched groups. Female adults (55±37) and young population (57±43) showed significantly low levels of iron in comparison to age matched males (adults: 81±54,$p=0.0007$ and young: 80±33, $p=0.006$). No significant difference was observed in calcium levels among all the groups.
Conclusion: Older population have significantly less deficiency of vitamin D, vitamin 12 and have low cholesterol levels. Male infants showed high prevalence of vitamin D deficiency and female infants showed high prevalence of iron and vitamin B12 deficiency. This study recommend supplementation of multivitamins to all breast fed infants regardless of being given formula feeds. This study also showed that prevalence of deficiency of these components changes within various age groups.

Introduction:

Iron and vitamin D are two essential nutrients which are required for growth & development and prevalence of deficiency implicate with adverse side effects. Vitamin D plays an important role in calcium and phosphate resorption, bone health and mineralization. Its deficiency results in a variety of skeletal, extraskeletal manifestations, depression, suicide, infectious diseases, Parkinson disease, autoimmune diseases, cancer, diabetes, heart diseases, obesity, chronic kidney disease hyperparathyroidism, rickets, osteomalacia, osteoporosis, and even fragility fractures(Aparna et al., 2018). Very few Indian studies have been published on the consequences of Vitamin D deficiency (VDD) leading to availability of most of the data from overseas studies. Many of the community based Indian studies have been conducted, however, most of them have been performed at different location with huge gap in age ranges (17-68) (Bachhel et al., 2015) and have focussed on specific population such as postmenopausal women(Marwaha et al., 2011).

Iron in particular is involved in many physiological processes, particularly in the production of red blood cells and myoglobin, oxygen transport and the production of ATP, DNA synthesis, and electron transport in mitochondria(Malczewska-Lenczowska et al., 2018, Abbaspour et al., 2014). Therefore, the lack of this mineral is associated with iron deficient anemia (IDA) (Clark, 2008). The women of reproductive age and children are particularly exposed to deficiency of this mineral because of heavy blood loss, pregnancy in women and exclusive dependency on breast feeding in children (Coad and Conlon, 2011, Milman, 2011). More than half of the world's undernourished population lives in India(Ganz, 2003). Although IDA occurs at all age and involves both the sexes(Kumari et al., 2017), there is a paucity of data about the age and sex wise epidemiology of anaemia in Indian population.

Indian population is more sensitive to vitamin B12 deficiency due to vegetarian food habit(Vecchio et al., 2014). Vitamin B12 is an essential vitamin that comes largely from non-vegetarian source. Deficient is related with irreversible and potentially severe nerve damage, depression, confusion, memory problems, and fatigue, constipation, loss of appetite, weight loss, face tremors in infants, feeding difficulties, irritation, and eventual growth problems if the deficiency is left untreated. However, none of these symptoms alone can diagnose vitamin B-12 deficiency. Data availability from different parts of country suggest replacement strategies and also on food fortification. However, data documenting age and sex wise status of vitamin B12 deficiency in India in general population is limited.

Hypercholesterolemia is very common among Asian population and it could be due to genetic risk, lifestyle factors , societal changes, lack of physical activity, and suboptimal dietary habits. The likelihood of dying from cardiovascular diseases in young people doubles with every 40 point increase in total cholesterol. Evidence is supporting the high incidence of heart diseases in developing countries in contrast to developed nations(Murray and Lopez, 1997). Although several reports and reviews documented the increasing prevalence of high cholesterol, and declining smoking rates among the educated Indians(Gupta et al., 2008a). All of these evaluations have multiple biases such as compilation of several studies from different sources and different methodologies(Ahmad and Bhopal, 2005).

In the present study, we evaluated the age and sex wise concentrations and prevalence of deficiency of vitamin D, vitamin B12, calcium, iron and cholesterol in Indian population. This study fills lacuna in literature and presents prevalence of deficiency of these components in Indian general population.

Materials and Methods

Population: In the present study levels of vitamin D (n=1423), cholesterol (n=793), calcium (1119), vitamin B12 (n=5608) and iron (n=341) was estimated in Indian population. Patients were categorized into 5 groups i) Infants (0-2 years) ii) children (3-12 years) iii) Teenager (13-18) iv) Young (19-35) v) adult (36-65) and vi) old (>65). Table 1 describe the number of patients in each group for vitamin D, cholesterol, vitamin B12, calcium and iron respectively. Table 2 demonstrate the normal range of each component.

Blood samples were collected from all the patients by taking aseptic precautions and transferred to serum vials. (Gel tubes-red cap). Once the blood is clotted, all the samples were centrifuged for 3000 RPM (revolutions per minute) for 5 minutes. The serum concentrations for vitamin D, iron, vitamin B12, cholesterol and calcium were determined by an automated analyzer (CX 9; Beckman, Brea, CA) with the use of commercial kits (Beckman coulter, CA).

	Infants (N)	Child (N)	Teenager (N)	Young (N)	Adults (N)	Old (N)	Total
Vitamin D (ng/ml)	131	155	139	1482	2100	227	1423
Vitamin B12 (pg/ml)	60	51	139	1748	3138	472	5608
Iron (ug/dL)	27	10	9	121	148	26	341
Calcium (mg/dl)	96	63	62	360	498	40	1119
Cholesterol (mg/dl)	0	0	14	199	527	53	793

Table 1: Total number of individuals in each age group

	Normal laboratory range as per the manufacturer recommendation
Vitamin D (ng/ml)	30-100 ng/ml (Aparna et al., 2018)
Cholesterol (mg/dl)	125-199 mg/dl
Vitamin B12 (pg/ml)	190-950 pg/ml
Calcium (mg/dl)	8.5-10.5 mg/dl (Harinarayan et al., 2007)
Iron (ug/dL)	Child 50-120µg/dl, Male 65-175µg/dl, Female 50-170µg/dl

Table 2: Normal range of each component

Results:

Lowest prevalence of vitamin D deficiency in older population

The mean 25(OH)-D concentration of the study group was 25.40 ± 13.20 ng/ml. Overall, 71% of the study population had a 25(OH)-D concentration <30 ng/mL, and were defined as having vitamin D deficiency. Old population (>65 years age) had highest vitamin D (29.93±19.61) levels than children: (22.94±15.09), Teenager (22.66±13.58), young (23.06±12.52), and adult (25.86±15.64); $p<0.05$; Figure 1) and low vitamin D deficiency than children (59% vs. 78%; $p=0.002$), teenager (59% vs.75%; $p=0.016$), young and adult population (59% vs 78%, $P=0.002$) except infants ($p>0.05$). The prevalence of vitamin D deficiency was highest in children and young groups (78%). Similarly, infants have significantly higher vitamin D concentration than children (27.99 vs. 22.94), teenager (27.99 vs 22.66) and young group (27.99 vs. 23.06) ($p<0.05$) (Table 1; Figure 1).

Next comparison was done in between males and females of each group. Female adults have significantly higher vitamin D concentration than age matched males (Males: 23.66±14; Females 26.46±15; $p<0.0001$). No significant difference in vitamin D concentration was found in males and females of other age groups. However, 76% of male infants showed vitamin deficiency in comparison to 62% of female infants ($p=0.03$).

	Infants	Child	Teenager	Young	Adults	Old
Vitamin D (ng/ml)	27.99 ±21.37 (N=131)	22.94±15.09 (N=155)	22.66±13.58 (N=139)	23.06±12.52 (N=1482)	25.86±15.64 (N=2100)	29.93±19.61 (N=227)
Deficiency of vitamin D (% population)						
Vitamin D	70	78	75	78	69	59

Table 1: Age wise distribution of vitamin D levels and deficiency in Indian population

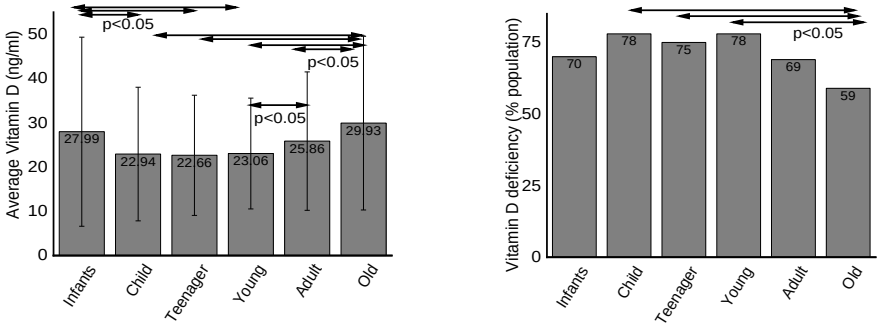

Figure 1: Graphical representation of vitamin D levels and deficiency in Indian population

	Infants			Child			Teenagers		
Vit. D (ng/ml)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	25.40±22.64 (N=60)	30.10±20.03 (N=71)	0.21	24.74±19.15 (N=64)	21.68±11.24 (N=91)	0.21	24.11±15.6 (N=41)	21.98±12.64 (N=98)	0.40
	Young			**Adult**			**Old**		
	23.91±12 (N=453)	22.69±12 (N=1029)	0.07	23.66±14 (N=754)	26.46±15 (N=1346)	**0.0001**	28.07±13 (N=138)	32.79±25 N=89)	0.06
Deficiency of vitamin D (% population)									
	Infants			**Child**			**Teenagers**		
Deficiency of vit D (% population)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	76	62	0.03	73	81	0.09	70	73	0.75
	Young			**Adult**			**Old**		
	76	79	0.73	75	68	0.34	62	53	0.25

Table 2: Age and sex wise distribution of vitamin D levels and deficiency in Indian population

Lowest prevalence of vitamin B12 deficiency in older population

47% of the population showed vitamin 12 deficiency (≤ 190 pg/ml). The mean concentration of vitamin B12 was 270±242. The prevalence of vitamin B12 deficiency was lowest in older population (32%) and highest in young group (60%) (P<0.05). Similar trend was seen in vitamin B12 concentration (Highest: Old-394±421; Lowest: Young-201±172). Old population (>65 years age; 394±421) had significantly high vitamin B12 (394±421) levels than Infants (296±279), children (238±149),Teenager (234±186), young (201±172) and adult (257±245); p<0.05; Figure 2) and low vitamin B12 (32%) deficiency than infants (40%), children (52%), teenager (50%), young (60%) and adult population (50%). In comparison to young population, adults have significantly higher vitamin B12 concentration. Whereas, female teenagers and adults have significantly high vitamin B12 levels in comparison to age matched males. However, male infants showed high vitamin B12 levels than female infants (p=0.005) with significantly low prevalence (30% vs. 48% respectively ;p=0.01) This data showed that female adults have significantly high vitamin D and vitamin B12 levels when compared with age matched males (Table3-4; Figure 2).

	Infants	**Child**	**Teenager**	**Young**	**Adults**	**Old**
Vitamin B12 (pg/ml)	296±279 (N=60)	238±149 (N=51)	234±186 (N=139)	201±172 (N=1748)	257±245 (N=3138)	394±421 (N=472)
Deficiency of vitamin B12 (% population)						
Vitamin B12	40	52	50	60	50	32

Table 3: Age wise distribution of vitamin B12 levels and deficiency in Indian population

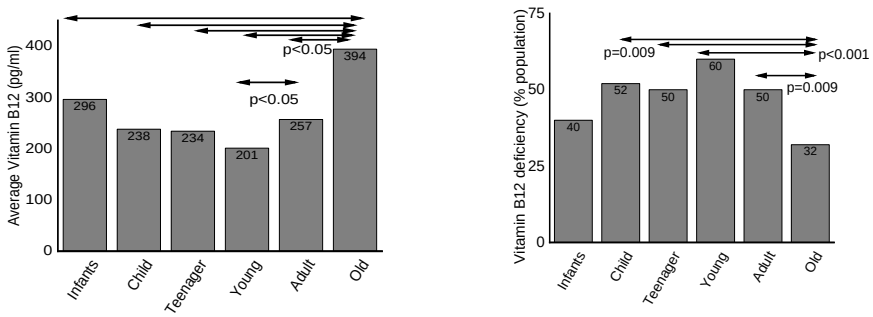

Figure 2: Graphical representation of vitamin B12 levels and deficiency in Indian population

	Infants			Child			Teenagers		
Vit. B12 (pg/ml)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	393±335 (N=26)	210±145 (N=34)	**0.005**	240±141 (N=19)	237±154 (N=32)	0.94	177±82 (N=37)	254±207 (N=102)	**0.03**
	Young			**Adult**			**Old**		
	193±157 (N=723)	208±182 (N=1025)	0.07	230±227 (N=1327)	277±256 (N=1811)	**0.0001**	375±398 (N=265)	425±456 (N=199)	0.21
Deficiency of vitamin B12 (% population)									
	Infants			**Child**			**Teenagers**		
Deficiency of vit B12 (% population)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	30	48	**0.01**	43	56	0.07	59	47	0.12
	Young			**Adult**			**Old**		
	62	58	0.66	55	45	0.16	34	30	0.54

Table 4: Age and sex wise distribution of vitamin B12 levels and deficiency in Indian population

Highest prevalence of iron deficiency in old population

71% of old males showed significantly high prevalence of iron deficiency (Female old: 50%, p=0.0003). Similarly female infants showed low iron levels (37±16 ug/dL), and high prevalence of iron deficiency (100%) when compared with males (78%; p=0.0001). Female adults (55±37) and young population (57±43) showed significantly low levels of iron in comparison to age matched males (adults:
81±54,p=0.0007 and young: 80±33, p=0.006). No significant difference was observed in calcium levels among all the groups (Table 5-7).

	Infants			Child			Teenagers		
Iron (ug/dL)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	62±63 (N=14)	37±16 (N=13)	0.17	61±50 (N=5)	33±18 (N=5)	0.27	27.6±6.4 (N=2)	56±42 (N=7)	0.39
	Young			**Adult**			**Old**		
	80±33 (N=33)	57±43 (N=88)	**0.006**	81±54 (N=57)	55±37 (N=91)	**0.0007**	67±58 (N=14)	67±58 (N=12)	1.0
Deficiency of iron (% population)									
	Infants			**Child**			**Teenagers**		
Deficiency of iron (% population)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	78	100	**0.0001**	60	66	0.46	100	57	**Not comparable due to n=2 in males**
	Young			**Adult**			**Old**		
	36	48	0.11	43	56	0.08	71	50	**0.0003**

Table 5: Age and sex wise distribution of iron levels and deficiency in Indian population

	Infants	Child	Teenager	Young	Adults	Old
Calcium (mg/dl)	8.8±1.4 (N=96)	9±0.86 (N=63)	8.9±0.8 (N=62)	9±0.9 (N=360)	8.9±0.83 (N=498)	9.1±1.1 (N=40)
Deficiency of calcium (% population)						
Calcium	21	19	27	21	20	20

Table 6: Age wise distribution of calcium levels and deficiency in Indian population

	Infants			Child			Teenagers		
Calcium (mg/dL)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	8.6±1.2 (N=64)	9.1±1.7 (N=32)	0.09	8.9±0.8 (N=32)	9.1±0.8 (N=31)	0.35	8.8±0.85 (N=18)	8.9±0.84 (N=44)	0.67
	Young			**Adult**			**Old**		
	9.1±0.98 (N=97)	8.8±0.83 (N=263)	**0.004**	8.8±0.86 (N=150)	8.9±0.80 (N=348)	0.2	9±1.02 (N=18)	9.2±1.1 (N=22)	0.56
Deficiency of calcium (% population)									
	Infants			**Child**			**Teenagers**		
Deficiency of calcium (% population)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	26	16	0.11	18	19	1.0	33	25	0.27
	Young			**Adult**			**Old**		
	16	23	0.28	22	20	0.86	22	18	0.59

Table 7: Age and sex wise distribution of calcium levels and deficiency in Indian population

Highest cholesterol level in Adults

25% of population showed high cholesterol level (≥200 mg/dl). Whereas we did not get any sample for infants and children group, all teenagers were having normal cholesterol levels. 31% of adults showed highest cholesterol levels in comparison to 18% of older population (p=0.04) (Table 9; Figure 4)). Similarly mean concentration of cholesterol was highest in adults (183±43; p<0.05)) in comparison to teenagers (Table 8; Figure 3). No significant difference was observed in other groups. Young females (48%) showed high prevalence of cholesterol when compared with age matched males (28%; p=0.0005) (Table 10; Figure 5).

	Infants	**Child**	**Teenager**	**Young**	**Adults**	**Old**
Cholesterol (mg/dl)	NA	NA	148±23 (N=14)	178±39 (N=199)	183±43 (N=527)	168±62 (N=53)

Table 8: Age wise distribution of cholesterol levels in Indian population

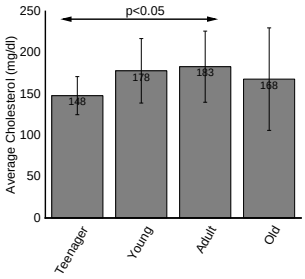

Figure 3: Graphical representation of cholesterol levels in Indian population

High cholesterol (% population)			
	Young	**Adult**	**Old**
Cholesterol (% population)	25	31	18

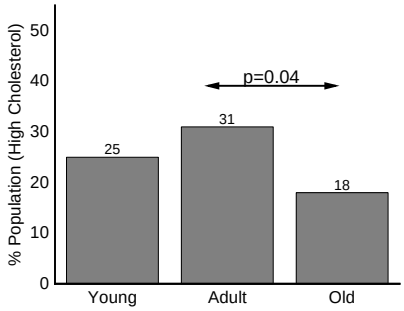

Table 9: % population showing high cholesterol levels

Figure 4: Age wise graphical representation of hypercholesterolemia in Indian population (%)

	Infants			Child			Teenagers		
Cholesterol (mg/dl)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	8.6±1.2 (N=64)	9.1±1.7 (N=32)	0.09	8.9±0.86 (N=32)	9.1±0.86 (N=31)	0.35	8.8±0.85 (N=18)	8.9±0.84 (N=44)	0.67
	Young			**Adult**			**Old**		
	9.1±0.98 (N=97)	8.8±0.83 (N=263)	**0.004**	8.8±0.86 (N=150)	8.9±0.80 (N=348)	0.2	9±1.02 (N=18)	9.2±1.1 (N=22)	0.56
High cholesterol (% population)									
	Infants			**Child**			**Teenagers**		
High cholesterol (% population)	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**	**Male**	**Female**	**p-Value**
	NA	NA		NA	NA		0	0	
	Young			**Adult**			**Old**		
	28	48	**0.0005**	28	34	0.44	20	17	0.71

Table 10: Age and sex wise distribution of cholesterol levels and hypercholesterolemia in Indian population

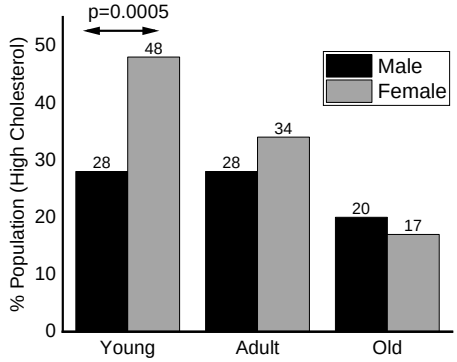

Figure 5: Age and sex wise graphical representation of hypercholesterolemia in Indian population (%)

Discussion

This study examined levels and prevalence of deficiency of vitamin D, vitamin B12, cholesterol, calcium and iron in Indian population. The community-based Indian studies of healthy controls reported a prevalence of VDD ranging from 50% to 94%. Estimated prevalence of vitamin D deficiency in 71% population agrees with the current understanding of status of Indian population.

Overall, we found that vitamin D deficiency was very common in Indian population of all age groups. Age dependent increasing prevalence of vitamin D deficiency has been reported by many studies(Jacques et al., 1997, Daly et al., 2012). It could be due to lower levels of 7-dehydrocholesterol, the precursor of vitamin D3 and therefore lead to VDD in older population (Holick et al., 1989). However, in the current study, prevalence was particularly low in old population and adults. Moreover, >70% of teenagers, children and a young age were deficient of vitamin D. Therefore, many factors must contribute to this phenomenon. The amount of sun exposure and vitamin D supplements could be a possible factor. Children, teenagers and young people spend most of their times indoors either due to education or work in India in contrast to elderly. Moreover, they may use more sunscreen creams because of cosmetic issues, spend more time on mobile games/internet surfing, television and therefore have less exposure to the sun or more prone to vitamin D deficiency.

Recent studies have reported VDD as being associated with various types of anaemia such as iron deficiency anaemia, one of the most widespread forms of anaemia in the world. Iron deficiency can lead to cognition defects, memory impairment along with impaired immune function, frequent infections and iron deficiency anaemia.

There is evidence that a deficit in iron may disturb the synthesis of vitamin D3 and lead to its mild deficiency, because it is an important co-factor for many enzymes like 1α-hydroxylase which is required for hydroxylation of 25(OH) D to 1,25(OH)2 D. Iron deficiency also impairs fat and vitamin A intestinal absorption. Therefore the absorption of vitamin D may also be impaired. This may contribute to the development of VDD. High prevalence of vitamin D and iron deficiency in old male population showed the age associated correlation of vitamin D and iron levels in this population. Out of the 27 children enrolled in the study 89 % were deficient in iron levels (<50 μg/dl) which agrees with the previous finding in Indian population. Iron deficiency during pregnancy and exclusive dependency of infants on breast feeding could be the leading causes of anaemia in infants and young children. Iron deficiency was more common in females of all age groups except in old age. In spite of the recommendation of iron and folic acid by Indian Government, the implementation of the programme is poor due to lack of logistic planning and liability(Kotecha, 2011). Our results are in agreement with other Indian studies

(Singh and Patra, 2014) and indicate that the iron supplementation programme for children aged ≤24 months should be better monitored with more focus on female infants(Iron deficiency: 100%).

Vitamin B12, mainly a dietary vitamin and deficiency of these are mainly in vegans or in individuals who have issues of absorbing the vitamins from the stomach/intestine. In a recent study adults have been shown to have higher risk of Vitamin B12 deficiency(Ryan-Harshman and Aldoori, 2008). However, in our study lowest prevalence of vitamin B12 deficiency in adults and highest prevalence in infants is seen. This could be again due to more consumption of vitamin supplements by older population and complete dependency of infants on breast feeding. Human breast milk contains low levels of vitamins and therefore, supplementation of multivitamins to all breast fed infants is necessary regardless of being given formula feeds. However, very few parents follow doctor's advice for vitamin supplementation as they believe that all nutrients are present in breast milk.

Cardiovascular diseases, especially coronary heart disease, are important public health problems in India and many developing countries(Gupta et al., 2008b, Gaziano, 2005). Increasing trend of these diseases due to high cholesterol is very common in India in contrast to developed countries where the incidence has decreased(Murray and Lopez, 1997). In our study 25% of the population have high cholesterol level and adults are having highest cholesterol levels in comparison to young and old population. However, we have not estimated levels LDL, HDL and triglycerides.

The strengths of this study are the large study population of all the age groups and comparison between males and females of age matched population. However, there are some limitations to the present study. Such as information about dietary intake of vitamin D, vitamin b12, iron, calcium and cholesterol, the amount of sun exposure, the amount of time spent outdoors, the use of sun-screen, subgrouping of population into urban/rural areas, food habits, occupation and seasonal variation is lacking. All of these factors could affect the levels of these components.

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