Significance of Vitamin D in health and disease is clear. Earlier it was known only for its crucial role in bone metabolism but off late it has been found that deficiency of this vitamin is associated with cardiovascular disorders, diabetes mellitus, malignancies and immunological disorders. Till a decade before it was assumed that Indian population is resistant to vitamin D deficiency but in a span of few years many studies sprouted up indicating that 50-90% of Indian people are deficient in vitamin D.

Aim – we have tried to estimate the levels of serum/plasma 25(OH) vitamin D values in people attending a tertiary care hospital for reasons other than frank vitamin D deficiency. Further we tried to study the impact of demographic variables like age, sex and religion on its levels.

MATERIALS AND METHODS – a one year cross sectional observational hospital based study was conducted out at Princess Esra Hospital Hyderabad during January 2014 – January 2015. Blood samples were collected aseptically from all 246 patients after an informed oral consent. Quantitative Elisa was performed on serum/plasma for 25(OH) vitamin D levels using commercially available kit from Calbiochem, Inc. USA.

Results – of the total 246 subjects tested 56% were females. Male to female ratio was 1:3. Individuals varied in age from less than 1 year to 95 years. Mean age of the subjects being 36.4±21.7 and mean vitamin D levels as 54.39±349.32. With respect to age deficiency was prevalent through all the age groups but more so in infants and very old followed by adults. Men were found to be more deficient as 57.35% for vitamin D. More number of females were insufficient and sufficient as 28.65% and 12.38% for vitamin D. Percentage of intoxication were almost equal in both sexes. Deficiency was common in Muslims. Insufficiency was evident in Hindus. Intoxication more in Christians.

Conclusion – the present hospital based study with random sampling technique does not provide us a clear idea of the vitamin D status of the subjects and estimation of vitamin D alone in absence of valuable indicators of bone health like serum calcium, bone mineral density and parathyroid hormone are misleading. Nevertheless it is better to supplement vitamin D in pregnant women, infants and very elderly group to avoid deficiency, related diseases and disorders.

INTRODUCTION
Vitamin D is a steroid hormone required for active absorption of calcium from intestine and in regulation of its homeostasis. Vitamin D2 is obtained from diet and Vitamin D3 is synthesized by the skin on exposure to UV light. The hydroxylated form of this vitamin D2 and D3 is called as 25 OH Vitamin D. It is present in circulation and is an accurate indicator of vitamin D status of the individual. Deficiency of vitamin D leads to skeletal disorders like osteoporosis, rickets, osteomalacia and osteoporosis. Off late it is implicated to be responsible for some malignancies, immunological disorders and cardiovascular diseases [1-3].

Over the years especially over the last decade many studies focusing on vitamin D deficiency in Indian population has sprouted up. Despite the fact that India is a tropical country lying between 8.4° and 37.6° north latitude and most of its regions receive ample amount of sunlight throughout the year with long duration of bright sunlight every day. Deficiency has been reported in all age groups and as per the international osteoporosis foundation report from north India 96% of neonates, 91% of healthy school girls, 78% of healthy hospital staff, 84% of pregnant women are vitamin D deficient [4, 5, 6]. In southern India it is reported as 40% among males and 70% among females with significant variations in vitamin D levels with respect to residence i.e urban or rural and occupation of the people [7].

India stands next to china in population census with diverse group of people in terms of complexion, food habits, environment and life style. All these factors influence the vitamin D levels in an individual. Therefore it is erroneous to assume that the whole population is deficient in vitamin D as the guidelines says that deficiency results when an individual vitamin D level falls below the 95th percentile of the normal population. Further we are using the recommended vitamin D levels set by international standards or kit inserts to define the status of an individual which is inappropriate. We need to carry out large cohort studies and establish population based reference levels before assigning an individual as vitamin D deficient. As over reporting and treating hypovitaminosis by giving vitamin D supplements and fortifying food with vitamin D may lead to intoxication which again creates health problems. Hence the ICMR has taken the initiative to perform large cohort study on Indian population.

AIM – we have tried to assess the basic levels of 25(OH) D in patients attending a tertiary care hospital for reasons other than frank vitamin D deficiency complaints. Further we have tried to assess the impact of age, gender and religion on it.

MATERIALS AND METHODS
A cross sectional hospital based observational study was conducted over a period of one year from January 2014 – January 2015 at Princess Esra Hospital of Deccan College of medical sciences to know the vitamin D status of the subjects attending a tertiary care hospital for reason other than frank vitamin D deficiency. After an informed oral consent two hundred and forty six patients’ blood was collected and serum analyzed for vitamin 25 (OH) D levels. The hospital is situated in the heart of the city Hyderabad with a diverse population in their life style, food habits, religion, skin complexion and occupation.

Inclusion criteria – subjects attending the outpatient and inpatient departments of various specialties with symptoms unrelated to frank vitamin D deficiency were included in the study.
Exclusion criteria – patients with chronic liver and kidney disease, congenital anomalies and malabsorption syndrome were excluded from the study.

25 (OH) Vitamin D levels were estimated using the Elisa kit from Cal biotech Inc. Cat# VD220B. CA 91978. The test is a quantitative solid phase competitive ELISA where 25 (OH) Vitamin D in patient serum or plasma competes with biotin vitamin D conjugate added simultaneously for binding sites on anti-vitamin D antibodies coated wells. The reaction is completed by addition of streptavidin HRP conjugate and finally TMB reagent detects the reaction.

Quality control – is achieved by running the test in duplicate and along with standards and controls provided by the kit.

According to Lips classification and Hillock MF et al the vitamin D status of an individual is defined as [8-12]

<20ng/ml = Deficient

> 20-30ng/ml = Insufficient

>30-100ng/ml = Sufficient

>100ng/ml = Intoxication

Further vitamin D deficiency has been characterized by Lips as severe, moderate and mild based on the levels of 25 (OH) D [13, 14].

< 5 ng/ml = Severe

5-10 ng/ml = Moderate

10-20 ng/ml = Mild

RESULTS

Of the total 246 subjects studied for 25 (OH) D levels, females outnumbered males by being 72%. The male to female ratios was 1:3. The age range of the subjects was from less than one year to 95 years. There was no significant variation in the mean age between the two genders, for men it was noted as 35.41 ± 25.2 and for females 36.8± 20.3. Majority of the subjects in the study were observed in the age group 41-50 year followed by 21-30 years and 31-40 years as seen in graph 1.

The mean 25 (OH) D levels were found to be 54.39 ± 349.32. In men it was it was noted as 26.20 ± 30.74 and for women 65.97 ± 414.26.

Table 1. Mean 25 (OH) vitamin D Levels with respect to age as is as follows:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Mean vitamin D3 levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>30.19±11.99</td>
</tr>
<tr>
<td>1-10</td>
<td>29.5±35.28</td>
</tr>
<tr>
<td>11-20</td>
<td>39.47±40.59</td>
</tr>
<tr>
<td>21-30</td>
<td>30.02±30.67</td>
</tr>
<tr>
<td>31-40</td>
<td>25.18±20.64</td>
</tr>
<tr>
<td>41-50</td>
<td>36.87±37.95</td>
</tr>
<tr>
<td>51-60</td>
<td>34.18±37.5</td>
</tr>
<tr>
<td>61-70</td>
<td>27.00±25.61</td>
</tr>
<tr>
<td>71-80</td>
<td>30.38±21.79</td>
</tr>
<tr>
<td>81-90</td>
<td>10.98±00</td>
</tr>
<tr>
<td>91-100</td>
<td>7.31±00</td>
</tr>
</tbody>
</table>

In table 1 we see that individuals in the age group 11-20 years had the highest mean levels for vitamin D and it is evident with few exceptions that with increasing age the vitamin D levels are gradually declining with lowest levels noted in the age group 81-90 and 90-100 years.

In graph 2 as per the recommended guidelines of national osteoporosis society April 2013, in the present study we found 123 of 246 subjects i.e.50% to be deficient for 25 OH vitamin D, with values <20 ng/ml in their blood. About 28% were found to be having sufficient levels of the vitamin D ranging between 30 -100 ng/ml. Nearly 11% of the subjects were found to be having insufficient levels of vitamin D with values being between 20- 30 ng/ml and another 11% were intoxicated with values above 100ng/ml.

In graph -3, as per the Lips classification of vitamin D deficiency into mild moderate and severe forms, we noticed that 48.36% of the studied subjects in the deficient group were having severe vitamin D deficiency with values less than 5 ng/ml, 22.95% were showing moderate deficiency with values between 5-10 ng/ml and 28.69% were having mild vitamin D deficiency with values between 10-20 ng/ml.

In graph 1, the age wise distribution of the subjects is as follows:
In graph 4 it is evident that individuals in all age groups right from infancy to 10th decade of life experience vitamin D deficiency with a significant $P$ value of 0.02 using chi square test from 2010 excel software. Cent percent individuals in the age group 81-90 and 91-100 years were deficient for vitamin D followed by infants as 68.42% then in the age group 21-30 years as 62.5% and 51-60 years as 50%, rest of the other age groups had less than 50% of the individuals as deficient. Vitamin D insufficiency is seen right from infancy till the 5th decade of life with maximum number of subjects in infancy as 21.06% followed by 51-60 years as 16.66%. People were found to have sufficient levels of vitamin D in the age range starting from infancy to 7th decade of life. With maximum percentage of subjects in the age group 71-80 years followed by 31-40 and 41-50 years. Intoxication was more evident in the age group 41-50 years followed by 11-20 and 51-60 years.

In graph 5 we see more number of males as 57.35% to be deficient for vitamin D than females 47.19%. Insufficiency is more common in females. And almost equal number of males and females are having sufficient levels of vitamin D and intoxication.

Graph 6 shows religion wise vitamin D status of the subjects studied. Vitamin D deficiency is more common in Muslim community followed by Hindus. Insufficiency is more in Hindus than in Muslims. Sufficient levels of vitamin D are seen more often in Hindus than in Muslims. Intoxication is more evident in Christians.

**DISCUSSION**

Several studies over the last decade has been performed to know the vitamin D status of Indian population and almost all have demonstrated 50-90% of the subjects as vitamin D deficient which is against the geographical norms [1,3,15,16-19]. In the present study as per the recent updates on vitamin D deficiency classification by national osteoporosis society [20] we found 50% of the subjects to be deficient in vitamin D which is equal to the hospital based studies reported by Rudrajit P. et al as 47.5% and 53.35% by Vishal R. et al [21, 22] and community based study reported by Sheik Adil from India as 52% [19]. But less when compared to the reports from community based studies by Harinarayan CV et al in urban adult population as 62% in males and 75% in females and in rural adult population as 44% in males and 70% in females [7].

The mean serum 25 OH D level of the studied subjects was 54.39 ± 349.32 which is higher when compared to other studies by Sheik Adil et al and Shah P. et al, Vishal R et al [22, 23, 27]. In the present study 10.97% of the individuals were found to be having insufficient levels of 25 OH D which is more when compared to reports by Marawah et al as 6.8% [2] Shah P. as 9.36% [27] and less when compared to reports by Rudrajit P. as 40% [21]. 25.6% by Sheik Adil [23], 19.48% by Vishal R [22].

We found only 28% of the studied subjects to be sufficient in vitamin D which is equal to reports by Vishal R [22] as 26.83% and is more when compared to reports by Rachna B. as 10% [28] and Rudrajit P as 12.5% [21].

Vitamin D deficiency in infants occurs when mother is deficient [18] and high prevalence has been recorded in infants with hypocalcemic seizures as 90%. Their mean serum 25 OH D levels were 6.5±3.22 ng/ml compared to healthy breastfed infants 9.06±4.78ng/ml [29]. Further in infants aged 6 months 16.49% developed rickets with mean 25 OH D values 16.96±13.33ng/ml [30]. In the present study it was seen in 68.42%. Vitamin D deficiency in elderly is common. The most likely reasons for it could be decreased dietary intake, diminished sunlight exposure, reduced skin thickness, impaired intestinal absorption and impaired hydroxylation of vitamin D in liver and kidneys [31]. In our case it is noted as cent per cent especially in the age group 81-90 and 91-100 years. Marawah et al from India noted it as 91.2% in elderly [26]. In the age group 21-30 years it is seen in 62.5% of the individuals followed by 50% in 51-60 years age which is similar to reports by Harinarayan CV and report by Rudrajit P. as 50% for the age group 21-30 years and 45% for the age group 41-50 years and 51% for the age group 51-60 years [21]. Highest prevalence by him was reported in the age group 31-40 years which is not seen in our case [25]. Rachana B. reported highest prevalence of 95% in the age group less than 31-40 years followed by 77% in the age group 41-50 years [28]. However, it is better to supplement vitamin D in infants or pregnant women and very old for better bone growth and bone health, to prevent rickets and osteoporosis and to reduce the associated risk of cardiovascular disease, malignancies and immunological disorders [27]. Vitamin D insufficiency is evident more in infancy and 51-60 years of age.
number of subjects in the age group 71-80 years followed by 61-70 years are sufficient in vitamin D which could be due to the fact that they might be receiving vitamin D and calcium supplements. Intoxication is more common in the age group 41-50 years and females which could be due the fact of aggressive vitamin D and calcium supplement therapy being instituted in females for fear of deficiency.

With respect to gender more number of males around 57% were deficient when compared to females 47%. This slight difference was noted by Sheik Adil too as 86% and 82% for levels less than 30 ng/ml [23]. But other authors reported it to be more prevalent in females than males as 92% by Rachana B. and 28 % in males, Rudrajit P. as 53% in females and 33% in males and Harinarayana CV as 63.2% in males and 75% in female seen in urban population and 44% males and 70% females in rural population [28,21,7]. This could be due to the possible fact that most of the urban males are involved in indoor jobs with little chance of exposure to sunlight and inadequate dietary calcium and vitamin D intake. Insufficiency is more evident in females which could be again due to diet poor in calcium and vitamin D, dress code use of sun screens and indoor life style. More number of females than males were found to be sufficient in vitamin D which could be due to females accounting for more number of study group. No statistical difference was noted in number of intoxicated individuals in both the sexes. Religion wise when we tried to correlate the finding more of the Muslims were found to be deficient than Hindus this is because the study group comprised of 96% of Muslims. More number of Hindus were found to have insufficient and sufficient levels of vitamin D than Muslims. Intoxication was more in Christians. This is due to fact that the proportion of the other religions in the study is meagre and findings do not give a real picture of vitamin D status.

LIMITATIONS

The study was performed as a hospital based study with no specific sample selection criteria, and vitamin D levels were estimated alone in absence of other important parameters like parathyroid hormone levels, serum calcium levels and bone mineral density which are essential and worthwhile indicators of calcium vitamin D parathyroid hormone axis. The study group comprised more number of females because of the reason that they had more complaints than males for which they visited the hospital and sought medical advice. Similarly more number of Muslims due to the geographical location. Hence sample size and distribution is not appropriate for generalizing the findings. To get a real picture of the vitamin D status of the population we need to carry out observational studies with large population size and case cohort studies to see the differences.

CONCLUSION

Vitamin D deficiency is prevalent in half of the subjects studied. Severe deficiency is seen in substantial proportion of the subjects studied. Around a quarter of subjects were having sufficient levels. The number of insufficient and intoxicated was almost equal. Intoxication is seen more in the age group 41-50 years and in females 75% which needs to be addressed to avoid hypervitaminosis D and its complications. All age groups are susceptible to vitamin D deficiency with more so the very old and infants with indication of vitamin D and calcium supplementation in this group. Men were found to be more deficient than females, which is contradictory to other studies published so far. Religion wise Muslims were found to be more deficient than people of other religions which could be due to dress code, indoor life style and malnutrition.

ETHICAL CLEARANCE – was obtained from the ethical committee of the Deccan College of medical sciences

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