25-Hydroxyvitamin D deficiency and Its Relationship To Obesity , Seasonal changes and Other Risk Factors In a Group Of Iraninan Children

Abstract: Serum 25-Hyroxivitamin D deficiency is an important problems of our new world ,which affects the growth and development of children in both developed and developing countries .There is increasing evidence of vitamin D deficiency world-wide resulting in nutritional rickets. With increasing use of fast foods and reducing appropriate physical activity, Our world is encountered with the problem of obesity which increases the risk of 25-hyroxy vitamin D deficiency. The aim of this study was to determine the status of serum 25(OH)D level in children 2-14 yr old who visited in out-patient pediatric endocrinology clinic between 1390-92. We also examined the relationships between serum 25(OH) D deficiency and BMI age ,sex, skin color ,season of laboratory measurement, sun exposure index and diary intake. using a cross sectional design ,serum 25 (OH)D level ,the amount of diary intake, BMI, BMI percentile and BMI Z score for age and sex, sun exposure index(= hours of sun exposure per week * fraction of Body Surface Area exposed to sunlight) , were measured in 170 children (2-14 yr old) living in Tehran. Our data was analyzed using Pearson correlation test ,linear regression test ,independent t test , chi 2 test and ANOVA test. All the children were divided into four groups according to their level of 25(OH)D .on the base of our review of literatures we use the cut off 30 ng/ml as the optimal level of 25(OH)D,20 ng/ml -30 ng/ml as 25(OH)D insufficiency,10 ng/ml -20 ng/ml as moderate deficiency and under 10 ng/ml as sever vitamin D deficiency.

The mean serum 25 (OH) D level was 21.37±11.54 ng/ml. and 23% of subjects had sever vitamin D deficiency (<10 ng/ml) and 78% of subjects did not have optimal 25 (OH)D serum level (<30 ng/ml). 25(OH)D levels were negatively and significantly correlated with BMI(r= -0.56 ,P value <0.0001),BMI percentile(r=-0.36, P value < 0.0001) , BMI z score (r= -0.41 Pvalue <0.0001)and age(r=-0.41 P value =0.001). 25 (OH)D level was positively and significantly correlated with diary intake (r= 0.68 P value <0.0001) and sun exposure index (r= 0.45 P value <0.0001).Prevalence of sever vitamin D deficiency was significantly higher among girls than the boys.(P value =0.01) and prevalence of sever vitamin D deficiency in subjects whose 25(OH)D were measured during summer was significantly higher than winter group(P value =0.03).Prevalence of sever 25(OH)D deficiency was significantly(P value <0.001) higher in obese group(BMI percentile for age and sex >95 and BMI Z score >1.5). over all we can conclude that our children specially obese ,girls, those who are in pre pubertal and pubertal age with rapid growth , are at high risk of 25(OH) D deficiency and its subsequent.

Key words: 25-Hydroxyvitamin D, season, sun exposure ,Children

Introduction:

25-Hydroxyvitamin D deficiency and obesity are two important problems of the new world, which affect growth and development of children in both developing and developed countries .There is increasing evidence of vitamin D deficiency World-wide resulting in nutritional rickets(1).Vitamin D is an important pro- hormone which regulates 3% of human genome(2),So it's deficiency may have wide ranging effects . The most frequent clinical presentations during childhood are skeletal complications. The prevalence of 25-hydroxyvitamin D deficiency world –wide is 30-80 % in children and adults(3).25-hyrdoxyvitamin D is the major circulating form of vitamin D, whose serum levels are the best available indicator of total body vitamin D status(4) .Vitamin D is a fat soluble vitamin with hormonal functions and it helps calcium and phosphorus homeostasis and bone metabolism. Exposure to sun light ,dietary intake and supplementation with vitamin D, are the main sources of this vitamin in human(5).solar UVB radiation penetrates the skin and converts 7-dehydrocholestrol to pre-vitamin D3,which ,in turn, converts rapidly to vitamin D3(6).The oily fishes ,egg yolks and fish oils contain vitamin D .Milk , cereals ,juices and yogurt are fortified in North America. The cutaneous synthesis of vitamin D may vary widely in different populations depending on their availability of sun exposure as well as actual sun exposure of the bare skin(1).skin pigmentation with melanin is a limiting factor in the cutaneous synthesis of vitamin D by as much as 99%(7,8).Hypovitaminosis D is recognized as a serum concentration < 30 ng/ml(9,10,11).Cut off point 20 ng/ml is used in some studies. The 2011 Institute of Medicine (IOM) in agreement with The Pediatric Endocrine Society defined serum 25-hydroxyvitamin D levels of >20 ng/ml (50 nmol/l) as the requirement in all children (1.6) ,but the cut off 30ng/ml is more acceptable ,because when 25-hydroxyvitamin D drops below 30-32 ng/ml ,calcium absorption is impaired and secondary hyperparathyroidism may occur and skeletal sequels of vitamin D deficiency may manifest. So it is evident that circulating levels of 25-hydroxyvitamin D of at least 30- 32 ng/ml are requires to optimize its functions(3,12,13). Nowadays we know obesity not only as a simple disease but also the most common and important risk factor of chronic disease such as diabetes, hyperlipidemia ,cancers, cardiovascular diseases and rickets and osteoarthritis .World Health Organization (WHO) reported that the global prevalence of obesity will be increased from 350 million and 1 billion over weight into 2.16 billion over weight and 1.12 billion obese in 2014.In Iran there are 24.5 million obese (2012) and over weight people which are almost 30 % of population (5,17,18).Obese children have poor diet habits and they are usually sedentary and less likely to play out doors, Therefore their exposure to sun light may be limited(19).Vitamin D insufficiency is highly prevalent in the world .It is estimated that > 1 billion people have vitamin D insufficiency .Even children who live in sub-tropical countries are at risk of vitamin D deficiency according tom recent studies(21).In this study we determine different risk factors of 25-hydroxyvitamin D deficiency ,such as season, BMI ,skin color, diary intake , age and gender.

Material and Methods:

Study population and data collection:

A cross-sectional study between years 1390-92 was used to determine 25-hydroxyvitamin D status and its risk factors, including age , gender, obesity, skin color ,diary intake and the season, in 170 children aged between 2 and 14 years old who presented to the out –patient clinic of pediatric endocrinology. 73 patients presented because of their parents' complaint of probable early puberty ,54 patients presented for over weight control.28 patients were presented because of their parents' complaint of inadequate growth, and 15 patients were presented just for routine check up .we excluded the children with rickets or hypocalcemia or those who had abnormal liver or renal or parathyroid functions .We also excluded those who had mal-absorption or celiac disease ,and those who used anti-convulsion or chorticho-steroid drugs .All the patients with genetic syndromes, diabetes and skeletal diseases were excluded(2,3). 25-hydroxyvitamin D was measured in all patients at Pars hospital laboratory using chemiluminescent immunoassay with Elexis 2010 cobalt E 411.Blood samples were kept in refrigerator for 30 minutes before centrifuge. Height was measured without shoes to nearest 1mm with a wall-mounted portable Seca 700 stadiometer (Seca gmbh & Co.kg), Weight was measured in a light clothing to the nearest 0.1 kg by Seca 700 (Hammer Steindamm 9-25) Germany-Hamburg.

We gathered the information about gender and age by using archive files of all 170 subjects .Then using their phone number we contacted their mother and asked about the diary intakes and their sun exposure. we considered one glass of milk or yogurt ,two glasses of dugh (churned sour milk) and 30 grams of cheese as one unite of diary .We estimated the amount of diary units each subject eat during one day(6,22,23,24).

Sun exposure was assessed using a telephone call and interviewing with patients' mother .Subjects were asked about their out-door activities over the previous week in terms of duration (in minutes) and frequency (per week).We use Lund and browder charts (25) and Nelson text-book of pediatric figure 68-3 (6) to determine the developmental percentage of BSA(body surface area) exposed to sun light by subjects attire during out door activity .The sun index ,Which is an index combining the measure of time out-doors during day light and BSA usually exposed to sun light during that time, was calculated as follows :

Sun index=(hours of sun exposure per week)*(fraction of BSA exposed to sun light)(8).

Statistical Analysis:

Statistical Analysis was done by the statistical package for the social sciences (SPSS-version 19).Means and standard deviation s were used to summarize continuous variable that were normally distributed .Kolmogorov –Smirnov test were used to check for normality of distribution. P value < 0.05 was considered evidence of non-normality .Statistically significant differences were tested for quantitative items by the independent t-test and ANOVA test, and for qualitative items by chi-2 test. The correlation between 25-hydroxyvitamin D and age , BMI Z score ,BMI percentile ,sun exposure index and diary intake were determined using parsons correlation test. A model using stepwise linear regressions was developed in order to determine the significant predictors of vitamin D. We obtained informed content from the parents' of children.25-hydroxyvitamin D was measured as a routin component of patients' diagnostic and therapeutic course.

Results:

We enrolled a total 170 children with mean age of 8.4 ±**3.4** years (range , 2- 14 years old).In our study population we have 91 girls and 79 boys .We classified our subjects into four groups according to their BMI percentile and Z-score : BMI percentile < 5 % as under weight ,BMI percentile 5-85 % as normal weight .BMI percentile 85-95% as overweight and BMI percentile >95% as obesity (3).We also classified BMI Z-score as follows: < -2 , -2 to 1 , 1 to1.5 and >1.5(21).Using the cut off 30 ng/ml , we classified our subjects into four groups according to their 25-hydroxyvitamin D level : < 10 ng/ml as sever deficiency, 10-20 ng/ml as moderate deficiency, 20- 30 ng/ml as mild deficiency or insufficiency and > 30 ng/ml as sufficient vitamin D level(9,21,26,27).The mean 25-hydroxyvitamin D serum concentration was 21.37 ±11.5 ng/ml.(range between 4 to 60 ng/ml).Median 25 –hydroxyvitamin D was 21.5 ng/ml. 23.5% of study population had sever vitamin D deficiency(<10 ng /ml).overally 78.2% had insufficient 25-hydroxyvitamin D level (< 30 ng/ml).

25-Hydroxyvitamin D and Age:

Mean serum 25-hydroxyvitamin D level in children aged between 2 to 6 year was 26.47 ± 10.93 ng/ml which was significantly(p value < 0.05) more than other age groups(6-10 and 10-14).Children aged between 10 to 14 year had the lowest serum 25-hydroxyvitamin D level (18.36 ± 9.90 ng/ml).Which was significantly lower than other age groups (pvalue < 0.001).Significantly highert proportion of children aged between 10 to 14 year had sever vitamin D deficiency compared to those aged between 2-6 year(30.5% vs 5 %, pvalue = 0.001).

25-Hydroxyvitamin D and Gender:

Mean serum 25-hydroxyvitamin D was significantly higher in boys compared to girls (23.56 ±11.52 ng/ml vs 19.46 ±11.27 ng/ml, p value =0.02).Prevalence of sever vitamin D deficiency was also significantly higher in girls compared to boys(pvalue =0.001).

25-Hydroxyvitamin D and Obesity:

Mean serum 25 –hydroxyvitamin D concentration was significantly lower in subjects with BMI-Z score > 1.5(obese group) than subjects with BMI –Z score between -1 to +1.5 (8.37 ± 2.38 ng/ml vs 27.09 ± 9.41 ng/ml , p value < 0.05).We determined that 26.5 % of our study population were obese and 14.1 % were over weight, Generally 40% of our study population did not have appropriate weight. The mean serum 25-hydroxyvitamin D level was significantly lower in those whose BMI percentile was more than 95%, than those with normal BMI percentile(5-85%) (8.29 ± 2.30 ng/ml vs 27.93 ± 9.72 ng/ml , p value < 0.0001).

25-Hydroxyvitamin D and Season:

We classified our study population into two groups according to the season of blood sampling for 25-hydroxyvitamin D measurement .We considered patients who were measured from first day of Farvardin up to first day of Aban (March 21st to October 23 th) as summer group and those who were studied between first day of Aban to the last day of Esfand as winter group (October 23th to March 20) (3,8).The mean 25-hydroxyvitamin D in summer group was not significantly higher than winter group (23.12 ±12.53 ng/ml vs 19.84 ±10.43 ng/ml. pvalue =0.06).But the prevalence of sever 25 –hydroxy vitamin D deficiency was significantly higher in winter group compared to the summer group(30% vs 16.5 % , p value = 0.03).

25-Hydroxyvitamin D level and Sun Exposure Index:

The mean amount of hours that subjects were exposed to the sun light during a hole week was 7.8 ± 4.11 hours.This was significantly lower in girls compared to the boys . (6.90 ±3.4 vs 8.9 ± 4.60 hours, p value = 0.02).

The mean amount of hours that subjects were exposed to the sun light during a hole week was significantly lower in obese group compared to those with normal BMI(p value = 0.001). We also determined that BMI and hours of sun exposure were significantly and inversely correlated (r= - 0.40 and p value < 0.0001).Our study also showed direct and significant correlation between 25-hydroxyvitamin D level and hours of sun exposure (r= 0.35 , p value =0.001).

Results was the same about SEI .The mean SEI was 2 ± 1.64 .SEI was significantly lower in girls than the boys(p value =0.0001).BMI and SEI were significantly and inversely correlated with each other (r= - 0.40 and p value < 0.0001)

25-Hydroxivitamin D and Diary Intake:

The mean unit of daily diary intake in our study was 1.83 ± 0.53 unit .The mean diary itake was significantly higher in those with normal BMI ,compared to obese patients (2.14 ± 0.42 vs 1.38 ± 0.39 , p value < 0.0001).

25-hydroxyvitamin D and diary intake were significantly and directly correlated (r= 0.68 , p value < 0.001).On the other hand diary intake and BMI were significantly and inversely correlated (r= -0.04 , p value < 0.0001).The mean diary intake during a day was significantly lower in those with sever vitamin D deficiency compared to those with vitamin D insufficiency of vitamin D sufficient subjects.

Our study showed significant and direct correlation between 25-hydroxyvitamin D and SEI ,hours of sun exposure and diary intake. But inverse and significant correlation between 25-hydroxyvitamin D and age ,BMI Z score and BMI percentile .Linear regression model also confirmed a significant inverse correlation between 25-hydroxyvitamin D level and age (pvalue < 0.0001. CI (95%) (-0.16 , -0.49).and a significant inverse correlation between 25-hydroxyvitamin D level and BMI Z score (p value < 0.0003, CI(95%) (-1.59, -

0.32).This test also showed a significant and direct correlation between diary intake and 25-hydroxyvitamin D level (p value <0.0001, CI (95%), (0.58-1.18).

The mean BMI level of our study population was 1.93 ± 4.58 kg/m2 . and mean BMI percentile and BMI Z score were 66.63 ±29.41 and 0.64 ± 1.15. There was no significant difference between genders according to their BMI. Mean BMI in children aged between 10 to 14 year was significantly higher than other age groups. The lowest amount of 25 –hydroxyvitamin D serum level was belonged to girls aged between 10 to 14 year(p value < 0.05).

Discussion:

Several studies examined the relations between serum concentration of 25-hydroxyvitamin D and varied health out comes ,and concluded that optimal serum concentrations are > 30 ng/ml(9,11). In this cross-sectional study , children aged 2 to 14 year.78.2 % of the children had low level of vitamin D concentrations .The prevalence of vitamin D deficiency was 83% during winter months ,like previous studies (9,11). The mean serum 25-hydroxyvitamin D level was 21.36 ± 11.5 ng/ml, which is lower than normal cut off (30 ng/ml).This shows that we are confronting with the problem of vitamin D deficiency. This problem is despite of abundant sun exposure like some other countries (28,13).Iran is locating in the latitude of 25 to 40 degree north, which can receive adequate sun exposure .But sedentary life style, air pollution, too many buildings and towers don't allow the sun light to penetrate to the skin. These results are also the same in tropic countries such as Saudi Arabia, Egypt, Emirates, Australia, Turkey , India and Lebanon, In these countries 30- 50% of children and adults have 25 –hydroxyvitam D level < 20 ng/ml, despite abundant sun exposure(29).Latitudes around 40 degree north or south have insufficient ultra violet B (UVB) radiation in winter time. Therefore in sunny countries especially in middle east such as Turkey (30),India (31) Kuwait (32), and Iran vitamin D deficiency which is common, is the result of cultural and religious believes(5).

But in other studies it had been concluded that the hours of sun exposure was more important than the body surface area which was exposed to the sun(8,11).

In our study the season was correlated with the level of serum 25-hydroxyvitamin D .In winter the prevalence of sever 25-hydroxyvitamin D deficiency was significantly higher compared to summer .This was similar to the other studies in America ,Canada ,United Kingdom and South Korea.(5,11,21,33)Any Caucasian Individual (like Iranians) in a bathing suit who is not tanned would receive a total body MED(minimal erythemic dose) from 10 to 12 minutes of peak July summer sun.For an Asian Indian that same MED could take 30 minutes of exposure and for a darkly pigmented African- American ,It would require 120 minutes of exposure to synthesize the same amount of vitamin D as a Caucasian exposed for 10 to 12 minutes.

The mean BMI level of our subjects was 19.31 ± 4.58 kg/m2 and mean BMI percentile and Z –score was respectively 66.63 ± 29.41 and 0.64 ± 1.50.

There was no significant difference between boys and girls according to their BMI. The mean BMI in children aged between 10 to 14 year was significantly higher than other age groups .The lowest amount of 25-hydroxyvitamin D was belonged to girls aged between 10 to 14 year ,and this was statistically significant(p value<0.050).

In other countries such as Saudi Arabia and Russia the mean 25-hydroxyvitamin D level was similar to our study(19.4 ng/ml and 21.16 ng/ml)(34,35).

With these evidences we can conclude that vitamin D deficiency is a world wide problem and that is important to study its risk factors. One of these risk factors is obesity .In our study there was a significant correlation between obesity and vitamin D deficiency .I n other studies in Poland, North-Eastern America and Malaysia this result was confirmed.(2,9,28).With increasing BMI Z score and percentile ,The mean serum 25-hydroxyvitamin D is significantly decreased and the prevalence of sever deficiency is significantly higher in obese children compared to those with normal BMI .In 2012 Harvard study.92% of obese children had serum 25-hydroxyvitamin D level < 30 ng/ml, This amount was 100% in our study .But in non-obese group In Harvard study 86% had 25 (OH)D level < 30 ng/m, and in our study this amount was 62.4 %.This difference may be because of exclusion of children aged between 2 to 6 year in Harvard study which were included in our study(14).In 2013 Saudi Arabia ,researchers found an inverse significant correlation between 25 (OH)D and BMI(r=-0.73,pvalue <0.01) (34).which was similar to our study (r=-0.56 ,p value <0.001).The reason of hypovitaminosis D among obese are wrong eating habits ,using too much fast foods and soda intake and diary elimination and its substitution with high caloric foods. Also sedentary life style and less out-door physical activity and less sun exposure will lead to more vitamin D deficiency(2,14,19,27,34).On the other hand because vitamin D is a fat soluble pro-hormone ,with increasing body fat mass ,large amount of vitamin D is sequestrated in fat tissue and its bioavailability will reduce(2,14,34).Many other researchers concluded that it is vitamin D deficiency which causes obesity .The association between hypovitaminosis D and hypertension ,hyperlipidemia and insulin resistance are studied ,It is found that vitamin D may have critical role in synthesis and secretion of insulin,by regulating plasma calcium level. So hypovitaminosis D can cause fat tissue accumulation(2,5,8).Some in-vitro investigations show that 25-OH-D inhibits the differentiation of pre-adipocyte and lipoprotein lipase,If this hypothesis comes true, vitamin D may be useful for weight control(5).For understanding the direction of causality between 25-OH-D and obesity ,a strong meta analysis were done and 21 adult cohorts (up to 42024 subjects) were reviewed by genotyping. On the basis of a bi-directional genetic approach that limits confounding factors ,That study suggests that a higher BMI leads to lower 25-OH-D level .While any effects of lower 25-(OH)D increasing BMI are likely to be small. This study showed that each 1 kg/m2 increase in BMI will cause 1.5 % decrease in 25(OH)D level (5).

Also our study showed that with increasing the age,25(OH)D level will decreased ,this result is confirmed in other studies(3,4,9,21,37).The mean 25(OH)D level is significantly higher in children aged between 2 to 6 year compared to those aged between 6 to 10 year and 10 to 14 year. This is acceptable because hypovitaminosis D is the disease of children in rapid growth period. In several studies the lowest level of 25-(OH)D was belonged to children aged between 11 to 16 year ,and excluding infancy period ,with increasing the age ,the level of 25-(OH)D was decreased(3).

Infants usually use multivitamin or human milk or formulas which is fortified with vitamin D, Although human milk in not a good source of vitamin D ,But taking multivitamins among infants is more common than older children.

Hypovitaminosis D is the disease of rapid growth and puberty period .In our study most of the children who presented in the clinic between 6 to 10 years old, were brought by their parents mostly because of the probable early puberty .And that may be the reason why the mean 25(OH)D level was lower in this age group compared to children aged between 2 to 6 year.

With increasing the age ,the tendency of using fast foods instead of diary, adolescents habits like using too much time sedentary with computers and parents less control on their children diet than before, will cause 25-OH D deficiency .Also with increasing the age ,the prevalence of obesity will increased .In our study 47% of our subjects aged between 10 to 14 year were obese ,and this was only 26% among children aged between 2 to 6 year .Like other studies ,in our study ,girls has lower level of 25 (OH)-D than boys, and prevalence of sever vitamin D deficiency was significantly lower in girls ,compared to boys.(5,27).

In south Korean study ,The difference between mean serum 25 (OH)D level between boys and girls was significantly only in adolescents(3).Our result was similar because we had the lowest serum level of 25-(OH)D level in girls aged between 10 to 14 year old. Because there was no significant difference between diary intake and BMI between two genders. This fact may be because of cultural and religious believes ,or less hours of sun exposure in girls(3).

The mean diary unit intake in our study was 1.8 unit per day .which is below the recommended level of 3 units per day .There was no significant difference between genders according to their diary intake. But the mean diary intake was significantly higher in children aged between 2 to 6 year. With increasing the age ,tendency to use high caloric fast food ,decrease diary intake .Although 25-(OH)D does not find in un-fortified diary ,but low diary intake is an indicator of not appropriate diet habits and obesity. Our study showed that in subjects with normal BMI, the mean diary intake was significantly higher compared to obese(2.1 units per day VS 1.38

unit per day , p value =0.01). In Texas study they showed that using fortified milk was significantly correlated with 25(OH)D level(p value < 0.004) and those who did not have enough milk intake, had a great intake of soda and fast foods and they usually skip breakfast(14). In America , Canada and Finland ,all of the diaries and cereal products are fortified with vitamin D(38). But in Iran few producers fortified their products, and these products are too expensive for normal population to use.

So we can conclude that sun exposure index, latitude, type of clothing, gender, age , obesity and diary intake are the most important factors that have effects on 25 –(OH)D serum levels. As our study and other studies show ,girls especially aged between 10 to 14 year and children aged between 6 to 10 year who are in rapid growth period , and obese children who did not have enough diary intake are at the highest risk of 25-hydroxyvitamin D deficiency.

By fortifying diaries and other food products with vitamin D and presenting these product with acceptable price, and changing diets and life style, taking at least 400 IU of vitamin D per day in all children, having sufficient physical activities and sun exposure ,we can control obesity and its effect on hypovitaminosis in our new generation. We will have healthy adults with less mosculo skeletal problems and we can reduce social and economic burden of obesity and hypovitaminosis D.

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