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Vitamin D Deficiency among Arab Community in North Israel: A Cross-Sectional Study

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Abstract

Objectives: Plethora of studies had described a global widespread vitamin D inadequacy. Studies have established that the prevalence of vitamin D deficiency is unexpectedly high in the Middle East. We aimed to determine the prevalence and the risk factors of vitamin D deficiency in Nazareth-Hospital Employees and Arab Football League.

Design: Population-based cross-sectional study was performed on 367 apparently healthy employees and 40 control football players. Serum levels of 25(OH)D, parathyroid hormone, calcium, phosphate and Body mass index were measured in summer. The LIAISON® 25 OH vitamin D assay uses immunoassay (CLIA) technology. Student's *t* test, Pearson *r* and one way ANOVA were used.

Results: Unexpectedly vitamin D deficiency was diagnosed on the basis of laboratory values in 91% of the hospital employees and in 72.5% of the football players (25(OH)D <30 ng/ml). The frequencies of deficiency (<20 ng /ml), insufficiency (20–30 ng/ml), and sufficiency (>30 ng/ml), were (59%, 32% and 9%) for employees and (25%, 47.5% and 27.5%) for players respectively. PTH and BMI results were 60pg/ml and 26kg/m2 for the employees and 38pg/ ml and 23kg/m2 for the players respectively. Comparing vitamin D, PTH values among Hospital employees versus football players the levels of vitamin D were significantly lower among hospital employees, whereas, the levels of PTH were significantly higher [(p<0.001 (95% CI -8.27 to -2.469) and [(p<0.001) 95% CI 10.15 to 23.9)], respectively. The hospital employees' correlation between (PTH and vitamin 5 D) and (BMI and vitamin D) were (r = -0.17; 95% CI -0.273 to -0.061, p=0.002) and (r = -0.2; 95% CI -0.3 to -0.09, p<0.001) respectively.

Conclusions: Vitamin D deficiency is a global health problem even in sunny climate. Long term strategies to address this issue should include public education, national health policies through food fortification, and vitamin D supplementation. Reappraisal of the range of the vitamin D level worldwide is warranted, and the need for reliable cutoff criteria to describe vitamin D deficiency is required.

Keywords: Vitamin D deficiency; Prevalence; Employees; Football players

Introduction

In the past decade, important steps forward the study of the authentic vitamin D pathophysiology have been made. In addition to its vital role in the bone homeostasis, skeletal development and maintenance, multiple facts are mounting, that vitamin D provides widespread beneficial effects, and not only on bone tissues, but to almost all tissues throughout the body and that the concentrations needed for optimal health are almost certainly higher than previously reported [1].

Simultaneously, vitamin D provides to the extra skeletal structures and the relatively high dominancy of insufficient levels of vitamin D have been largely unknown by both clinicians and patients [2].

Chemically all forms of vitamin D are secosteroids share a close structural and functional resemblance to steroids. Fundamentally, the term vitamin D indicates to a pair of biologically inactive precursors of a critical micronutrient. They are vitamin D2 or Ergocalciferol or 25-hydroxyvitamin D [25(OH)D] and vitamin D3 or Cholecalciferol or 1,25-dihydroxyvitamin D [1,25(OH)2 D][3].

Vitamin D2 is produced by the action of UV-B- irradiation on ergosterol, a 5,7-diene phytosterol, which is synthesized by fungi and phytoplankton but not in the animal kingdom [4]. It is the major form of dietary vitamin D in humans[5] and its bioavailability from UV-B-irradiated and vitamin D boosted mushrooms, revealing a new promising approach of improving the vitamin D maintenance in the general population[6,7].

Vitamin D3 is produced with peak synthesis occurring between

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295 and 297 nanometers [8] and this is the form that goes to work by attaching itself to vitamin D receptors present throughout the body. Vitamin D is acquired both through nutritional means (10-20%) and by the cutaneous synthesis under the action of sunlight (80-90%) [9].

Vitamin D has multifunctional activities with pleiotropic effects on calcium and phosphorus metabolism, hence it's vital role in the bone homeostasis, and in safeguarding against an array of diseases acts in concert with other nutrients and hormones to support healthy bone renewal - an ongoing process of mineralization and demineralization which, when awry, shows up as rickets in children and osteomalacia or osteoporosis in adults [10-18].

Although there is no consensus or some disagreement on optimal levels of 25(OH)D as measured in serum, vitamin D deficiency is defined by most experts as a 25(OH)D below 20 ng/mL and vitamin D insufficiency as a 25(OH)D level of 21 ng/mL to 29 ng/mL [9,19-23].

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Page 2 of 7

Deficiency of vitamin D can be attributed to different reasons: It can result from inadequate nutritional intake coupled with inadequate sunlight exposure and other disorders that limit vitamin D absorption and impair the conversion of vitamin D into active metabolites.

The growing understanding of the multifunctional roles of vitamin D throughout the body has provided new insights and shed lights on the function of this vitamin.

It has been estimated that 1 billion people worldwide are affected by various degrees of vitamin D deficiency [24]. This global concealed epidemic phenomenon is more harmful than previously thought, rationalize the serious attention attributed to this vitamin [25].

Despite the plentiful sunlight year round in the Middle East (15°-36°N), it has one of the highest rates of vitamin D deficiency worldwide.

One of the first studies was conducted in university students and elderly from Saudi Arabia, and revealed a mean 25(OH)D level ranging between 4- 12 ng/mL [26] The mean 25(OH)D level was near 10 ng/ mL in Lebanese, Saudi, Emirati and Iranian women. [27-29] A similar mean was recorded in elderly Lebanese [30], Kuwait [31] and the United Arab Emirates (UAE) [32].

It was 35% for a vitamin D level below 10 ng/mL in a study of elderly subjects from a geriatric hospital in Israel [33] and between 60-65% in Lebanon, Jordan and Iran [34], and was 48% for a cut-off less than 15 ng/mL in subjects from Tunisia [35]. Hovsepian et al. from Isfahan city, Iran, and Bener et al. from Qatar reported a high prevalence of vitamin D deficiency (50.8%)[36] and (68.8%)[37] respectively.

In France, 14% of healthy adults have serum 25(OH)D concentrations below 12 ng/mL [38], and 29% in Finland [39]. Some population subgroups may be at greater risk of vitamin D deficiency, e.g., the elderly and subjects with darker skin pigmentation or subjects living in regions with low levels of sunlight. Reported prevalence levels within high risk groups rises to 47% amongst elderly people in Europe [40] and 42% amongst Afro-American women in the USA [41]. Because the scarcity of population data on vitamin D status in Israeli Arabs, the objective of this study was to conduct an unique novel study to assess the prevalence of vitamin D deficiency among the Nazareth-E.M.M.S Hospital Employees and among professional football Arab players living in the same metropolitan area in north Israel.

Methods

This current population-based cross-sectional study was performed during the summer of 2011 after obtaining signed written informed consent at the study enrollment from all participants. 367 (60% females) apparently healthy employees and 40 control healthy males' football players recruited to undergo a blood test, nutritional and activity assessments during the summer period (Table 1).

Venous blood samples were collected into plain tubes, and serum was separated and stored at -70°C until analysis. The hospital employees were divided in subgroups according to their profession as follow: 46(12.5%) Physicians [mean age \pm SE = 47.5 \pm 1.25]; 182(49.5\%) Nurses [mean age \pm SE = 40.25 \pm 0.89] and 139(38%) Administration [mean age \pm SE = 40.27 \pm 0.89] (Table 1). The football players were divided according to their team location as follow: 7(20%) from Nazareth town [mean age \pm SE = 22.5 \pm 4.69]; 20(50%) from Sakhnin town [mean age \pm SE = 24.8 \pm 5.3] (Table 1). Complete physical examination for every subject was performed and serum levels of 25-hydroxy vitamin D (25(OH)D), parathyroid hormone (PTH), calcium, phosphate, Body mass index (BMI), and nutritional intake, and lifestyle variables that constitute

potential risk factors for vitamin D deficiency were evaluated. The LIAISON 25 OH Vitamin D TOTAL Assay uses chemiluminescent immunoassay (CLIA) technology for the quantitative determination of 25-hydroxyvitiamin D and other hydroxylated vitamin D metabolites in human serum was used for the assessment of vitamin D levels. Assay results were used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions in an adult population. The subjects were classified as vitamin D-deficient, -insufficient, or -sufficient on the basis of 25(OH) D concentrations of < 20 ng/mL, 20-30 ng/mL, and > 30 ng/mL, respectively, according to recent consensus [43].

Descriptive results are presented as mean±SE. Student's *t* test was used to compare the differences between the hospital employees and the football player's subjects. We used Pearson *r* to determine the correlation between 25(OH)D and PTH levels and to characterize other relationships among variables. One way ANOVA multiple comparison tests were used to compare vitamin D levels and age. *P* values < 0.05 were considered significant.

Results

A total of 407 subjects were evaluated during the study (Table 1). The mean \pm SE age (in years) for hospital employees' subjects versus the control football players' subjects was 42.67 \pm 0.96 versus 23.3 \pm 4.58 (p<0.05). Of the total number of hospital employees surveyed, 26 out of 367 subjects (7%), are under medications for chronic diseases [(13/26 have Hypertension); (7/26 have type 2 diabetes mellitus and hypertension) and (6/26 have type 2 diabetes mellitus)]. Using one way ANOVA Nonparametric Turkey's multiple comparison test, no significant differences were observed between the chronic diseases and vitamin D levels (p>0.05) (Table 2).

We found a significant higher vitamin status in football players (n=40) compared to the total healthy employees (n=341) after the

| | Hospital Employees subgroups | | | |
|---------------|------------------------------|-----------------------|-----------------------------|--|
| N° (%) | Physicians 46 (12.5%) | Nurses 182 (49.5%) | Administration 139 (38%) | |
| Age Mean ± SE | 47.5 ± 1.25 | 40.25 ± 0.75 | 40.27 ± 0.89 | |
| Females # (%) | 7 (15%) | 136 (75%) | 77 (55%) | |
| Males # (%) | 39 (85%) | 46 (25%) | 62 (45%) | |
| | Football Team | s subaroups | · | |
| N° (%) | Nazareth 7 (20%) | Sakhnin 20 (50%) | Arrabeh 13 (30%) | |
| Age Mean ± SE | 22.5 ± 4.7 | 24.5 ± 4.1 | 24.8 ± 5.3 | |

| Table 1: Demographic | data of the | participants. |
|----------------------|-------------|---------------|
|----------------------|-------------|---------------|

| | Vitamin D (ng/ml) | PTH (pg/ml) | BMI (kg/m ²) |
|-------------------|-------------------|-------------|--------------------------|
| Employees (n=367) | 19.47 ± 0.4 | 59.9 ± 2.5 | 26.2 ± 0.2 |
| Phys. (n=46) | 21.27 ± 1.1 | 60.97 ± 3.3 | 27.1 ± 0.2 |
| Nurses (n=182) | *17.93 ± 0.7 | 61.1 ± 2.5 | 25.8 ± 0.3 |
| Admin. (n=139) | *20.9 ± 0.6 | 58.05 ± 5.1 | 26.3 ± 0.5 |
| Players (n=40) | 24.84 ± 1.2 | 37.80 ± 2.3 | 23.2 ± 0.2 |
| Nazareth (n=7) | 24.10 ± 2.4 | 31.84 ± 4.1 | 23.07 ± 0.6 |
| Sakhnin (n=20) | 23.95 ± 1.4 | 39.44 ± 3.4 | 23.5 ± 0.2 |
| Arrabeh (n=13) | 26.61 ± 2.5 | 39.08 ± 4.3 | 22.7 ± 0.2 |

Table 2: The table shows the (mean±SE) of the vitamin D, PTH and BMI for the total Employees and their subgroups [(Physicians, Nurses and Administration and for the football players and their subgroups resident at (Nazareth, Sakhnin and Arrabeh)]. The table also shows a statistically significant difference in the level of vitamin D in favor of the subgroups of Administration employees (20.9 ± 0.6) versus the levels of vitamin D among Nurses (17.93 ± 0.7). The statistical significant difference is marked by red star (*), p<0.05)].

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exclusion of those who carry chronic diseases [(n=26), (p=0.0003)]. In addition, when we compared the levels of vitamin D of the healthy employees (n=341) with the diseased employees (n=26), there was no statistical significant differences between the two groups (p=0.76). Additionally, females employees (220/367) were divided according to the traditional religious uncovered head versus covered head hair (200/220 versus 20/220), respectively. Females employees with covered hair had statistically significant lower vitamin D status compared to those with uncovered hair (95% CI-12.2 to 3.84, p<0.001), (Figure 1). Hospital employees were also divided to nonsmokers versus smokers (288/367 vs 79/367) respectively, the levels of vitamin D were significantly higher among smokers (95% CI 0.063 to 4.474, p=0.04) (Figure 2). The football players' subjects do sportive activity starting their day at 10.00am and performing outdoor football games at stadium at least 5 hours a day with their face, legs, arms, and forearms exposed to sunlight without using sun cream. Suboptimal vitamin D status observed in 91% of the hospital employees and in 72.5% of the football players (25 (OH)D <30 ng/ml), (Figure 3). The frequencies of

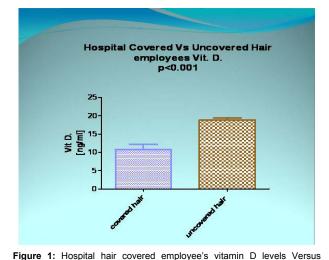
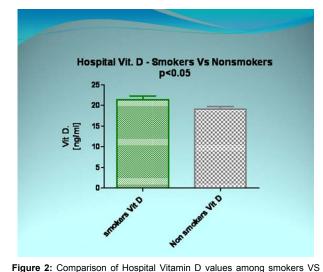
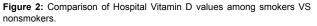
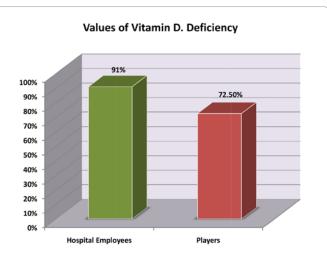
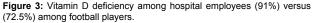


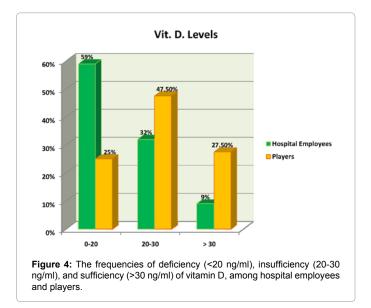
Figure 1: Hospital hair covered employee's vitamin D levels Versus uncovered hair employees.











deficiency (<20 ng /ml), insufficiency (20–30 ng/ml), and sufficiency (>30 ng/ml), were divided as (59%, 32% and 9%) for employees and (25%, 47.5% and 27.5%) for players respectively, the differences were statistically significant [(p<0.001 (95% CI -8.27 to -2.469)] (Figure 4). In comparison with football players and employees vitamin D levels versus age, there was no significant correlations (p=0.972), (p=0.84) respectively.

Body Mass Index (BMI) ranges [normal range (18.5-25)] were 26 units for the employees versus 23 units for the players (Table 2). Comparing the vitamin D deficiencies, PTH values between employees vs. players, the values were statistically significant and pronouncedly evident among employees in comparison with the players values [(p<0.001 (95% CI -8.27 to -2.469) and [(p<0.001) 95% CI 10.15 to 23.9)] (Figure 5 and Figure 6). PTH values; [normal values (10-55pg/ml)]; were 60pg/ml for the employees versus 38pg/ml for the football players (Table 2). The difference of the prevalence of subjects with secondary hyperparathyroidism (value >55pg/ml) between employees and players was calculated (Figure 7). The result demonstrates that (17.5%) of players and (38%) of hospital employees have PTH values

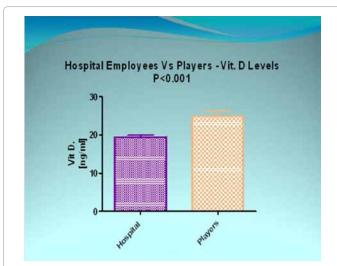
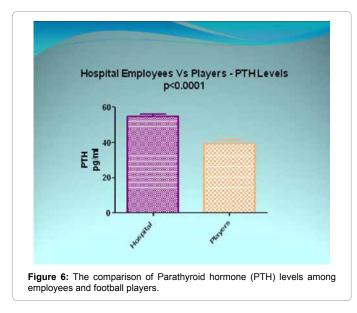


Figure 5: Hospital employee's vitamin D levels among employees and players.



up to 55pg/ml. This result shows a clinically relevant portion of subjects with vitamin D deficiency. Indeed, a secondary hyperparathyroidism based on low vitamin D status mean a real clinical sign for a deficiency. The hospital employees' correlation between (PTH and vitamin D) and (BMI and vitamin D) were (r = -0.17; 95% CI -0.273 to -0.061, p=0.002) and (r = -0.2; 95% CI -0.3 to -0.09, p<0.001) respectively, (Figure 8 and Figure 9).

Discussion

To the best of our knowledge, this is the first epidemiological report of prevalence of vitamin D deficiency among professional Arab medical employees and football players in Israel. The novelty of the current study is the unexpectedly high prevalence of vitamin D deficiency [(employees vs. players); (91% vs. 72.5%)] respectively. The overstate assumption that football players and professional medical employees may have normal values of vitamin D was refuted by our study. In fact, it was astonishing to find low concentrations of 25(OH)D in healthy hospital employees in a country with ample sunshine.

Smokers who spend more than five minutes under the sunlight

have higher levels of vitamin D comparing to non smokers who stay indoors (p<0.05). Moreover, a statistical significant effect of vitamin D levels were found in favor of uncovered head versus covered head hair (p<0.001). Our results concur with other previous studies that have inverse correlation between BMI and vitamin D deficiency [10,11]. Indeed, vitamin D plays an important role in diverse physiologic functions such as bone homeostasis [10,11]. Therefore, the likely disturbances due to vitamin D deficiency and the therapeutic potential of vitamin D were expanding [13-17].

Page 4 of 7

Determining vitamin D status can be a challenging task. Defining different levels of vitamin D according to health consequences rather than population means is the preferred method but has led to an ongoing debate about what constitutes "normal" or "optimal" 25(OH) D levels. Several criteria have been used to define sufficient 25(OH) D levels, including the level associated with optimal suppression of circulating PTH levels, greatest calcium absorption, highest bone mineral density (BMD), lowest rates of bone loss, falls, or fractures [43].

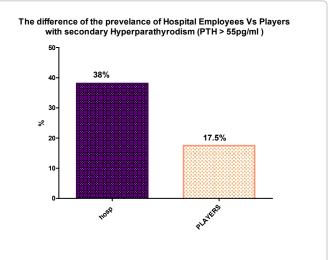
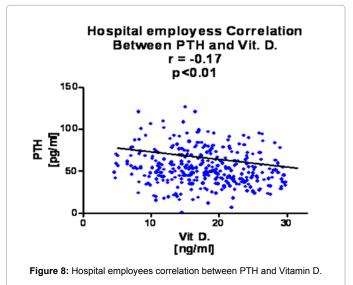
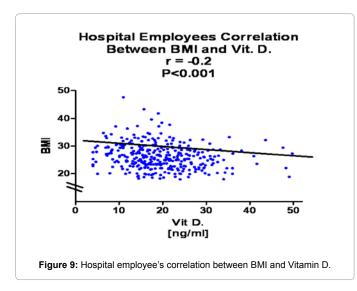


Figure 7: The figure shows the difference of the prevalence of subjects with secondary hyperparathyrodism (PTH>55pg/ml) between employees and players. It demonstrates the clinically relevant portion of employees and football players with vitamin D deficiency (38% vs 17.5%), respectively.





Until the 1990s, the criterion for appropriate vitamin D nutrition was simply the absence of overt rickets or osteomalacia [44]. Now, circulating 25- 16 hydroxyvitamin D [25(OH)D] concentrations are the appropriate measure of vitamin D nutritional status.

It is wrong to assume that simply because individuals live at southern latitudes, they need less vitamin D supplementation-some people actively avoid exposing skin to the sun, and the supply of dermal vitamin D is a function of sun exposure, amount of skin surface exposed, and dark skin pigmentation.

In a study by Plontikoff et al. [45] in their Minnesota-based study had deficient levels of vitamin D on patients with non-specific musculoskeletal pain and it was found that 100% of African Americans, East Africans, Hispanics, and American had deficient levels of vitamin D<20 ng/ml. Vasikaran et al. [46] found that 34% of blood donors age 18-67 years in Australia had vitamin D of <20 ng/ml. In a study by Tangpricha et al. [47] they observed that 36% of young adults are vitamin D deficient reflecting that wide distribution of vitamin D deficiency even among young people. Vitamin D deficiency is common in the Middle East. Woodhouse et al. [48] reported a low vitamin D level in Saudi Arabia population despite sunny days are almost all year round. In recent study by Sadat-Ali et al. [49] they found 25-37% of healthy Saudi men have vitamin D deficiency.

This study was conducted on healthy professionals including physicians, nurses, administrations and professional football players. The mean age was 40.5 years vs. 23.8 respectively. The study revealed that 91% of hospital employees and 72.5% of the players have vitamin D <30 ng/ml. This is an extremely high prevalence of vitamin D deficiency among young individuals with no musculoskeletal complaints. Participants with low vitamin D level were called for counseling and treatment. Very few studies conducted on health care professionals are reported in the literature.

During 2007-2008 a similar study was performed among health care staff at Hamad Medical Corporation in Doha, Qatar, it was observed that (96.5% of the study sample had vitamin D level of <30 ng/ml, 87% had vitamin D level of <20 ng/ml, while 54.7% had severe deficiency with a level of <10 ng/ml, and 20% had vitamin D below the lower limit of detection [3ng/ml]) [50]. In Boston Medical Center, it was observed that 32% of healthy students, physicians, and residents were found to be vitamin D deficient, despite adequate nutrition regime [51]. Another study conducted by Jancin on 35 internal medicine house staff at Oregon Health Sciences University, Portland, revealed that 51.4% of them were vitamin D deficient [52]. In a study on pregnant and lactating women who were thought to be immune to vitamin D deficiency since they took a daily prenatal multivitamin containing 400 IU of vitamin D (70% took a prenatal vitamin, 90% ate fish, and 93% rink approximately 2.3 glasses of milk per day; (73% of the women and 80% of their infants were vitamin D deficient (vitamin D level, <20 ng per milliliter) at the time of birth [53].

Only 5% of our hospital employees reported an average daily time spent outdoor of more than hour and their vitamin D did not differ from others who reported spending less time outdoors.

This could be because they are spending this time in the shade indoor or not getting enough exposure to the sun. In sports medicine vitamin D deficiency has become a hot topic recently. This study helps to show that it is a problem not limited to elderly or less healthy patients. It is a problem among professional athletes, such as National Football League (NFL), as well. In accordance with the levels of our NFL results of vitamin D deficiency, our NFL (n=40, mean age = 24y), from northern Israel showed that 72.5% - had abnormal vitamin D levels of less than 30ng/mL. 10 players had deficient levels (< 20 ng/mL) and an additional 19 players had levels consistent with insufficiency (20-30ng/mL). 11 players had values within normal limits (> 30ng/ mL). Comparable vitamin D levels were reported from 89 professional football players (mean age = 25y) from the New York National Football League (NFL) team in the spring of 2010 found an alarming percentage of players - 80.9% - had abnormal vitamin D levels, of less than 32 ng/ ml. 27 players had deficient levels (< 20 ng/mL) and an additional 45 had levels consistent with insufficiency (20-31.9 ng/mL). 17 players had values within normal limits (>32 ng/mL).

In addition, all players sustaining injuries that caused them to miss at least 1 practice or game had vitamin D levels that were significantly lower than players without muscle injury. Among the 18% of players who sustained a muscle injury in the previous season, all had statistically significant lower vitamin D levels, compared with those without muscle injury. There were no other statistically significant differences between those who did and did not sustain the injuries. The mean vitamin D level among players who did not sustain a muscle injury was 24.7 ng/ mL (range, 9.0 to 46.0 ng/mL), and the mean level among the 16 players who did suffer a muscle injury was 19.9 ng/mL (range, 8.0 to 33.0 ng/ mL) (P < .04) [54]. In contrary, among the Arab Football League no muscle injury was registered. All our football players were in good health condition and participate in all the practice and games.

A subsequent study examined the data on vitamin D insufficiency in 98 athletes and dancers (age range 20 to 30 years) in a sunny country. 73% of the participants had vitamin D insufficiency, and insufficiency was greatest among dancers and basketball players (94% in each group) [55,56]. In consensus with other studies, our study demonstrates high prevalence of vitamin D deficiency among our NFL and showed no link to an increased rate of muscle injury. This research joins other international findings pointing to a need to examine and address vitamin D insufficiency and deficiency not only in football players, but in other athletes as well. In conclusion, vitamin D deficiency is a global public health problem across all life stages with deleterious immediate and latent manifestations. Long term strategies to address this silent disease should include public education, national health policies for screening whole population and prevention through food fortification, and treatment through vitamin D supplementation. In addition, reappraisal of the range of the vitamin D level globally is warranted. Furthermore, we stress the need for a reliable cutoff criteria to describe

ISSN: 2161-0509

Page 6 of 7

vitamin D deficiency in the south of the Equator, since single cutoff value may be suitable for populations living in sunny climate with dark skin with relatively insufficient production of vitamin D or – viceversa – but might be inappropriate for others living in shadowy climate with bright skin with high production of vitamin D,(since more than 80% of vitamin D is produced in the skin by a photoreaction on exposure to ultraviolet B light from the sun).

Summary

Our aims were to conduct a novel study to assess the prevalence of vitamin D deficiency among hospital employees and among professional football players living in the same metropolitan area in Israel whose geographical alignment includes latitude of 31° 30' N, and 13 hours of sunlight in summer and 10 hours in winter, taking into account the impact of different variables; such as biochemical measurements, traditions, environmental risk factors on the development of vitamin D deficiency, in an area with plentiful sunlight throughout the year, and the strategies that should be implemented to prevent and treat this deficiency. High vitamin D deficiency was diagnosed in 91% of the hospital employees and in 72.5% of the football players on the basis of laboratory values. When comparing vitamin D, PTH values among Hospital employees versus football players the levels of vitamin D were significantly lower among hospital employees (p<0.001), whereas, the levels of PTH were significantly higher (p<0.0001). In the light of these findings we have proposed to discuss whether the reassessment of the cutoff criteria already used to describe vitamin D deficiency worldwide, is adequate for warm climate. In addition, the high prevalence of vitamin D deficiency among the above-mentioned populations raises the question of whether vitamin D supplements and food fortification should be given to these groups on a routine basis even in a sunny country such as Israel.

Strengths and Limitations

To the best of our knowledge, the current study is the first to investigate and compare the prevalence, biochemical measures, customs, environmental risk factors on the development of vitamin D deficiency among hospital employees and professional football players in Israel. This study also brings forth clearly the low dietary calcium intake and the limited exposure to sunlight of both the hospital employees and football players, which requires a need for screening and supplementations of vitamin D for both groups, even to those who live in sunny countries, because this may be a simple way to help prevent injuries and maximize muscle function, especially among athletics. The study was cross-sectional, and that limits the generalizability of these results. Furthermore, there are methodological limitations in this study since our sample of hospital employees and football players are a sample of convenience, more subjects in all age groups of both sexes in urban and rural locations in different parts of the country should be studied in the future. In addition, the strikingly high prevalence of vitamin D deficiency in this population necessitate radiographic assessments, another biomarkers of vitamin D status, in a subset of patients such as football players to detect their bone demineralization, considering the fact that all deficiencies were subclinical.

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Page 7 of 7

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