Adaptive Neuro-Fuzzy Inference System-Based Exploration of the Interrelationships of 25-Hydroxyvitamin D, Calcium, Phosphorus with Parathyroid Hormone Production

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Abstract The rationale of the current study was to assess the prevalence of 25-hydroxyvitamin D (25-OHD) deficiency and hyperparathyroidism in South Indian population and to explore interrelationships of 25-OHD, Ca, P towards parathyroid hormone (PTH) production using adaptive neuro-fuzzy inference system (ANFIS). A total of 407 subjects (228 men 179 women) with the mean age 56.8 ± 14.1 were tested for these parameters. In view of the skewed distribution of biochemical variables, data segregation was performed in tertiles and this data was trained to generate fuzzy interference system based on subclusters. The optimized model had 358 nodes and followed 44 fuzzy rules for prediction. This ANFIS model demonstrates that the deficiency of 25-OHD and Calcium triggers PTH production. PTH elevation is significant when Phosphorus is in the highest tertile. The associations observed by this model were consistent with the Kendall-Tau correlation matrix, which revealed inverse associations of Ca with P; and Ca with PTH and positive associations of P with PTH, and Ca with 25-OHD. Furthermore, the association statistics of the machine learning algorithm were also consistent, which suggested that depletion of Ca below 8.245 mg/dl was shown to elevate PTH levels

greater than 167 pg/ml when $P > 4.66$. Subnormal depletion in 25-OHD (9.3–16.2 ng/ml) is associated with subnormal elevation in PTH (47–73.6 pg/ml). To conclude, ANFIS and machine learning algorithm are in agreement with each other in stating that 25-OHD deficiency triggers lower calcium levels, lower calcium and higher phosphorus trigger PTH production.

Keywords 25-Hydroxy vitamin D · Parathyroid hormone · Calcium · Phosphorus · Machine learning

Introduction

Industrialization and urbanization resulted in lesser exposure to UV-B radiations, which in turn prevented the cleavage of B-ring of 7-dehydrocholesterol present in basal keratinocytes thus contributing to deficiency of vitamin D3 [1]. Vitamin D-binding protein produced by epithelia facilitates the transit of vitamin D3 to the liver to undergo hydroxylation at position 25 [2]. Second hydroxylation step occurs at position 1 in the kidney thus forming 1,25(OH)₂ vitamin D3 in the presence of 1-alpha-hydroxylase [3]. Parathyroid hormone (PTH), Interferon (IFN)-gamma and insulin-like growth factor 1 (IGF-1) were reported to induce while Ca^{2+} , P_i and klotho were reported to inhibit the activity of 1-alpha-hydroxylase [4]. PTH is secreted by the Chief cells of the parathyroid gland and regulates the distribution of total body calcium ions. The calcium-sensing receptor (CaSR) is activated by circulating Ca ions and represses PTH release from the Chief cells. When circulating Ca levels reduce below a threshold, CaSR signalling is reduced resulting in PTH release. PTH receptor (PTHr1) is highly expressed by osteoblasts and osteocytes and when PTH gets ligated, increases intracellular Ca [5].

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Vitamin D deficiency was reported to be 66.7% in Indian infants and 81.1% in Indian mothers [6]. A recent study demonstrated 90% prevalence of 25-OHD deficiency in women with the polycystic ovarian syndrome, which is correlating with higher BMI, lower HDL and Ca, and elevated testosterone [7]. Recently, we reported that 25-OHD deficiency increases the risk for diabetes and renal disease by 3.03-folds and 2.09-folds, respectively [8]. The total serum 25-OHD levels were reported to have a positive association with bone mineral density at L1-L4, femoral neck and total hip; and inversely associated with PTH [9]. Subjects with PTH levels in the highest tertile were reported to exhibit 3.09-folds higher risk for cardiovascular death compared to subjects with PTH in the lowest tertile [10].

In view of the very high frequency of 25-OHD deficiency reported in Indians and multi-disease association of 25-OHD deficiency, we have planned to assess the prevalence of hypovitaminosis D and hyperparathyroidism and to explore the interrelationships of 25-OHD, Ca, P in PTH production using adaptive neuro-fuzzy inference system (ANFIS) and machine learning tools.

Materials and Methods

Recruitment of Subjects

A total of 407 subjects (228 men and 179 women) in the age group of 15–93 year were enrolled in the study. Their mean age was 56.8 ± 14.1 year. The study protocol was approved by the Institutional Ethical committee of Nizam's Institute of Medical Sciences, Hyderabad. Informed consent was obtained from all the subjects. The recruitment took place in the outpatient unit of Nizam's Institute of Medical Sciences, Hyderabad during the period of December 2016 to January 2018. Subjects suffering from infections, inflammatory diseases, auto-immune disorders are excluded from the study. Participants using vitamin D supplements were excluded.

Biochemical Evaluation

Fasting blood samples are collected in plain vacutainers and serum separated was used for the biochemical analysis. Calcium and phosphorous levels were estimated by using Roche Cobas c501 fully automated chemistry analyzer (Roche Diagnostics, U.S.A.) using 5-nitro-5'-methyl-BAPTA (NM-BAPTA) and ammonium molybdate methods, respectively. Intact parathyroid hormone (PTH) and Vitamin D total assays are performed on ADVIA Centaur® XP Immunoassay system using kits manufactured by Simens Healthcare Diagnostics Inc., U.S.A that utilize

sandwich immunoassay and competitive immunoassay, respectively.

Development of Adaptive Neurofuzzy Inference System (ANFIS)

In view of the skewed distribution of biochemical variables, raw data is converted into tertiles and ANFIS model was generated using 25-OHD, Ca and P as input variables and PTH as the output variable. Subclustering was performed by fixing the range of influence as 0.5, squash factor as 1.25, accept ratio as 0.5 and reject ratio as 0.15. The hybrid method that integrates both neural network and fuzzy logic principles was adopted for training. The optimized ANFIS model was based on a total of 44 fuzzy IF–THEN rules with 358 nodes.

Machine Learning Algorithm Levels for Association Statistics

In order to establish an association between two attribute-value pairs called antecedent (*Ant*) and consequent (*Con*), support and confidence were calculated based on below-mentioned formulae. Let '*a*' is the number of data points that fulfill *Ant* and *Con*, '*b*' the number of data points that fulfill *Ant* but do not fulfill *Con*, '*c*' the number of data points that fulfill *Con* but do not fulfill *Ant*, and '*d*' the number of data points that fulfill neither *Ant* or *Con*.

$$\text{Support} = (a)/(a + b + c + d)$$

$$\text{Confidence} = (a)/(a + b)$$

Machine learning tools use top-down approach to identify the associations whose support and confidence are above the defined thresholds of *minconf* and *minsup*.

Statistical Analysis

The distribution of continuous variables in terms of kurtosis and skewness is estimated to determine the sharpness of the peak and whether the distribution is normal, right-handed skew or left-handed skew. The range of the data and mean and SD were calculated using the standard formulae. In view of the skewness of distribution, the data was segregated into tertiles based on the percentile distribution. This categorized data was computed in 2×2 contingency table (highest tertiles vs. lowest tertile for two variables) and Fisher exact test was performed to calculate odds ratio (OR) and 95% confidence interval (CI). Kendall-Tau correlation matrix was generated to evaluate the correlations between the variables. All the statistical tests were performed using the computational webpage www.statpages.info.

Results

In the current study, 50.1% subjects had the 25-OHD deficiency (< 20 ng/ml). The Ca levels in the deficient group were significantly lower compared to subjects with 25-OHD sufficient group (8.79 ± 1.10 mg/dl vs. 9.03 ± 0.97 mg/dl, $p = 0.03$). No such differences were observed in Serum P levels (3.95 ± 1.53 mg/dl vs. 3.92 ± 1.33 mg/dl, $p = 0.83$). PTH levels were > 65 pg/ml in 43.0% subjects.

As shown in Table 1, the distribution of age showed mild skewness. Skewed distribution was observed for Ca, P, PTH and 25-OHD. In view of the skewed distribution of biochemical variables data was segregated into tertiles as mentioned in Figure Legends.

As shown in Fig. 1, Calcium showed an inverse association with phosphorus ($p < 0.0001$) and PTH ($p < 0.0001$) while it showed a positive association with PTH ($p = 0.02$). Phosphorus showed a positive association with PTH ($p < 0.0001$). As shown in Fig. 2, association

Table 1 Demographic characteristics of studied population

Variable	Range	Mean	SD	Kurtosis	Skewness	Histogram
Age (year)	15–93	56.8	14.1	− 0.26	− 0.23	
Men: women	228:179					
Calcium (mg/dl)	1.0–12.0	8.9	1.1	9.21	− 1.77	
Phosphorus (mg/dl)	1.4–11.1	3.9	1.4	4.14	1.73	
PTH (pg/ml)	1.2–1171	129.7	192.1	9.43	2.93	
25-OHD (ng/ml)	1.0–383.0	26.6	27.8	66.68	6.07	

Scatter Plots and p-values

Fig. 1 Kendall-Tau correlation matrix. This matrix revealed inverse association of Ca with P and PTH while demonstrating positive association of Ca with 25-OHD and P with PTH

Fig. 2 Association statistics among Ca, P and PTH. When calcium levels are depleted below 8.245 mg/dl, PTH levels were found to increase. When 25-OHD levels are between 9.3 and 16.2 ng/ml, PTH levels will be between 47 and 73.6 pg/ml

statistics showed the when calcium levels go below 8.245 mg/dl and phosphorus > 4.66 mg/dl, PTH levels were greater than 167 pg/ml. When 25-OHD levels are between 9.3 and 16.2 ng/ml, PTH levels will be between 47 and 73.6 pg/ml.

The ANFIS model depicted that when 25-OHD and Ca are in the lowest tertiles, PTH will be in the highest tertile (Fig. 3). When 25-OHD is in the lowest tertile and P is in the highest tertile, PTH will be in the highest tertile (Fig. 4). When Ca is in the lowest tertile, P is in the highest tertile, PTH will be in the highest tertile (Fig. 5).

Fig. 3 Influence of 25-OHD and Calcium in inducing PTH production. In1: 25-OHD in tertiles (1: 1.0–14.2 ng/ml; 2: > 14.2–26.8 ng/ml; and 3: > 26.8–383.0 ng/ml); in 2: Calcium in tertiles (1: 1.0–8.7 mg/dl; 2: > 8.7–9.3 mg/dl; and 3: > 9.3–12.0 mg/dl); out1:

PTH in tertiles (1: 1.2–40.1 pg/ml; 2: > 40.1–84.3 pg/ml; and 3: > 84.3–1171.0 pg/ml). Significant elevation in PTH was observed when 25-OHD and calcium are in the lowest tertiles

Fig. 4 Influence of 25-OHD and Phosphorus in inducing PTH production. In1: 25-OHD in tertiles (1: 1.0–14.2 ng/ml; 2: > 14.2–26.8 ng/ml; and 3: > 26.8–383.0 ng/ml); In 3: Phosphorus in tertiles (1: 1.4–3.2 mg/dl; 2: > 3.2–4.0 mg/dl; and 3:

> 4.0–11.1 mg/dl); out1: PTH in tertiles (1: 1.2–40.1 pg/ml; 2: > 40.1–84.3 pg/ml; and 3: > 84.3–1171.0 pg/ml). Significant elevation of PTH was observed when 25-OHD is in the lowest tertile and Phosphorus is in the highest tertile

Fig. 5 Influence of Calcium and Phosphorus in PTH production. In 2: Calcium in tertiles (1: 1.0–8.7 mg/dl; 2: > 8.7–9.3 mg/dl; and 3: > 9.3–12.0 mg/dl); In 3: Phosphorus in tertiles (1: 1.4–3.2 mg/dl; 2: > 3.2–4.0 mg/dl; and 3: > 4.0–11.1 mg/dl); out1: PTH in tertiles (1:

1.2–40.1 pg/ml; 2: > 40.1–84.3 pg/ml; and 3: > 84.3–1171.0 pg/ml). Significant elevation in PTH was observed when Calcium is in the lowest tertile and Phosphorus is in the highest tertile

Discussion

Our results are in agreement with Yu et al. [11] in demonstrating a positive association of 25-OHD with Ca and inverse association of 25-OHD with PTH. The positive association of PTH with phosphorus and inverse association of PTH with 25-OHD were consistent with the observations of Vikrant et al. [12] although that was on patients with the chronic renal disease while the current study is population-based. Corroborating with the study of Souberbielle et al. [13], no gender influences were observed on the 25-OHD levels. However, age 60 year or more was shown to be associated with 1.99-folds risk for 25-OHD deficiency while our study showed no such association. This anomaly could be due to unreported vitamin D supplementation in few subjects. Seasonal variations, specifically higher 25-OHD and lower PTH levels were reported in summer compared to winter in both the genders [14]. However, cyclical variations in bone turnover rate were more pronounced in women compared to men [14]. Bjorn Jensen et al. [15] developed and validated a vitamin D status prediction model by considering dietary and supplementary vitamin D intake, outdoor physical activity, tanning bed use, smoking and month of blood draw as the predictors and explained 40.1% variance in 25-OHD among Danish pregnant women. The current ANFIS model demonstrates how 25-OHD can influence bone mineral metabolism with respect to Ca and P thus regulating PTH production. The interrelationships deduced from this model strongly support the existing literature where in vitamin D deficiency was reported to lower Ca and P absorption from the intestine thus lowering serum Ca

levels [16]. In response to low Ca, PTH is secreted, which increases mobilization of Ca and P from the bone [17].

In subjects with long-term peritoneal dialysis, Ca > 9.5 mg/dl and P > 6.5 mg/dl or < 3.5 mg/dl were reported to have an association with increased mortality while PTH has a less prominent effect on mortality [18]. Among the lifestyle risk factors, smoking was shown to influence PTH levels with current smokers having lowest PTH compared to never smokers [19]. The subjects with 25-OHD deficiency were reported to have elevated total cholesterol and low-density lipoprotein (LDL)-cholesterol than those with 25-OHD sufficiency [20]. The 25-OHD levels were reported to be positively associated with bone mineral density at the hip and spine; while serum PTH inversely associated with BMD at the hip alone in men [21].

The major strength of the current study is the application of ANFIS and machine learning tools to delineate the interrelationships of age, gender, Ca, P, PTH and 25OH. This helped in establishing the threshold of Ca and P that trigger PTH elevation. However, lack of data on bone mineral density is a limitation that needs to be addressed in future studies.

To conclude, South Indian population has a high incidence of 25-OHD deficiency (50.1%) and hyperparathyroidism (43.0%). Positive association of Ca with 25-OHD and P with PTH; and inverse association of Ca with PTH and 25-OHD with PTH highlight the interrelationships between these variables. Application of machine learning tools showed that Ca < 8.245 mg/dl elevates PTH > 167 pg/ml when P > 4.66 mg/dl. The ANFIS model demonstrated that 25-OHD deficiency results in low serum Ca; low calcium and high phosphorus trigger PTH production.

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Compliance with Ethical Standards

Conflict of interest All the authors hereby declare no conflicts of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Institutional Ethical committee of Nizam's Institute of Medical Sciences approved the study protocol.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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