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Evaluation of Serum 25 - Hydroxy Vitamin D and Zinc in Patients of Female Pattern Hair Loss (FPHL)

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ABSTRACT

Introduction
FPHL stands for Female Pattern Hair Loss, the most common cause of diffuse thinning of hair in females which sometimes lead to significant impairment in their quality of life. Genetic predisposition, iron deficiency and hormonal imbalances are established factor for FPHL but association of serum 25-hydroxyvitamin D [25-(OH) D] and zinc with FPHL is still under study.
Objective
The aim of this study was to evaluate serum 25-(OH) D and zinc levels in women with FPHL.
Methods
This was a case control study done in the department of Biochemistry at IGIMS Patna, Bihar. Serum 25-(OH)D and serum zinc were estimated in 50 patients of FPHL of age group 16-45 years and compared with 50 healthy females of same age group, same socio economic status and history of almost equal exposure to sunlight as in case group.
Conclusion
Low serum 25-(OH) D and zinc might play a role in pathogenesis of FPHL.

KEYWORDS FPHL, 25-hydroxyvitamin D, zinc

Introduction:
FPHL is a progressive non scarring and diffuse alopecia presenting with reduction in hair density mainly over the frontal and vertex areas but parietal and occipital regions may also be involved(1).

Miniaturization of hair follicles resulting from a progressive reduction of successive hair cycle time is pathogonomic of FPHL (2).

There is conversion of terminal hair follicles into vellus-like follicles which have a shortened hair cycle as their anagen phase is reduced and produces hair shafts that are small and thin(3). About 12% women aged between 20 and 29 years are affected by FPHL which increases to 41% by 69 years, and over 50% by 79 years(4).

It has been established that 60-70% cases of FPHL develop in individuals with no clinical symptoms or biochemical report of androgen excess (5). Also FPHL has been diagnosed in women with no circulating androgen (6). So the term Female Pattern Hair Loss and Female Androgenic Alopecia are used differently. (7)

It has been proven that vitamin D is necessary to delay the process of aging, including hair loss (8).Moreover vitamin D helps in the absorption of zinc.(9)

So vitamin D deficiency may present with hair loss because of its role in delaying the aging phenomenon and helping in absorption of zinc.

Zn deficiency manifests as many skin disorder including alopecia. (10, 11).

Acrodermatitis enteropathica a manifestation of zinc deficiency also present with hair loss (11).

Women with FPHL develop feeling of low self esteem, lack of confidence and restrict social contact due to perception of decreased attractiveness. (12)

So seeing the association of vitamin D and zinc with alopecia we evaluated the serum level of these two parameters in FPHL patients.

Vitamin D deficiency is defined as serum level of 25-(OH) D level below 20 ng/ml and insufficiency < 30 ng/ml (13).

The reference range for serum zinc level is 70-120 µg/dl (10.7-18.4 mmol/L), and value below 70 µg/dl is considered as deficient (14).

Material and methods:
This was a case control study that was carried out in Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna, Bihar from august 2015 to June 2016.Patients with complain of hair loss were viewed by expert dermatologist and only those patients were enrolled who had hair loss in the form of FPHL.50 such patients of FPHL were taken who were in age group of 16-45yrs .Control group consists of healthy females, paramedical staffs and female volunteers in the age group of 16-45yrs and of same socioeconomic status and history of almost equal exposure to sunlight. These 50 patients of FPHL were graded by expert dermatologist according to the Ludwig scale findings as mild, moderate & severe. Ludwig scale classify hair loss in females according to severity into three stages: type I (mild), type II (moderate), type III (severe) (15).

Inclusion criteria:
Newly diagnosed and confirmed cases of hair loss due to FPHL.

Exclusion criteria:
Patients with any systemic or scalp disease related to hair loss. Patients on drug that cause hair loss like antidepressant, anticonvulsants etc. Patients showing symptoms of hyperandronism like PCOD, hirsutism etc. Patients taking vitamin or mineral supplements. Serum 25-(OH) D was estimated by chemmiluminescent immunoassay, Beckman Coulter Access2. Name of the reagent kit was Access 25(OH) Vitamin D Total Reagent Pack with Cat. No.A98856.

The estimation of 25-(OH) D by Access 25(OH) Vitamin D Total assay occurs in dual step. In the first incubation reaction, sample along with DBP(vitamin D binding protein) releasing agent and paramagnetic particles coated with sheep monoclonal anti-25 (OH) vitamin D antibody is added. Vitamin D is released which binds with monoclonal anti-25 (OH) vitamin D antibody. In the second incubation reaction 25-(OH)D analogue-alkaline phosphatase conjugate is added which competes for binding with monoclonal anti 25(OH)vitamin D.Unbound material are washed away while material bound to solid phase are held in magnetic field. Lumi-phos 530 which is a chemiluminescent substrate is added and light generated is measured with a luminometer, which is inversely proportional to the concentration of 25-(OH) vitamin D in the sample (16).

Serum zinc was estimated by colorimetric kit method. Zinc binds to a ligand with development of absorbance at 560 nm (17). Written informed consent was taken from all subjects before undergoing any intervention. 5 ml blood was collected by venipuncture under standard precaution. It was allowed to clot before centrifugation and serum was separated as soon as possible. Serum 25-(OH) D and zinc was estimated by their respective methods. This serum sample can be kept stoppered and stored at room temperature for 72 hours only.

The internal and external quality control was performed in laboratory for all parameters done there. Hemolyzed samples were discarded .The serum samples were repeatedly assessed to confirm the value obtained.

Statistical analysis
Statistical analysis was performed using Graph pad Prism (version 5.0). Data obtained were presented as mean ± SD. One way analysis of variance (ANOVA) was applied to the result data of different groups of FPHL patients. Results of the study were discussed at 95% confidence interval. Interpretation of the test results was done according to p value. ($p < 0.05$ is significant).

Result:
This study enrolled a total of 100 female subjects of which 50 were patients of FPHL and 50 were normal controls. The age of participants in both study groups ranged from 16 to 45 years. The mean age of FPHL patients was 30.26±7.86 and that of control was 29.78±8.00. There was no statistical difference in age between the two groups (p=0.76).

These 50 patients of FPHL were graded by senior dermatologist according to the Ludwig scale as mild (type I), moderate (type II) or severe (type III) .Out of 50 FPHL patients 32 were mild , 15 were of moderate, and 3 were of severe Ludwig type. Serum level of 25-(OH) vitamin D and zinc were significantly decreased among cases as compared to control with p=0.0001 and p=0.001 respectively as shown in table I. Values of serum level of 25-(OH) D and zinc were compared among mild, moderate and severe group of FPHL patients by one way analysis of variance (ANOVA). Serum level of 25-(OH) D showed a significant decrease in level with increase in severity or grade of FPHL with p=0.01 as shown in table II. Serum level of zinc showed no significant correlation with grading of FPHL.

Table I- Comparison between cases and controls with respect to serum 25-(OH) D and serum zinc

Parameters	Case group (n=50)	Control group (n=50)	P value
Serum 25-(OH) D (ng/ml)	17.66±8.34	41.23±16.96	0.0001
Serum zinc (µg/dl)	69.92±12.71	80.76±19.32	0.001

Parameter	FPHL patients (n=50)			F	P value
	Mild (n=32)	Moderate (n=15)	Severe (n=3)		
Serum 25-(OH) D (ng/ml)	20.12±8.97	13.70±4.76	11.29±4.83	4.52	0.01

Table II- Relation between Ludwig Degree and mean 25-(OH) D among FPHL patients

Discussion:
Although FPHL is not a life threatening disease but has been reported to be associated with various psychological symptoms of depression , low self esteem due to cosmetic disruption as compared to MPHL(male pattern hair loss)(18). So prompt diagnosis, treatment & supplementation is essential for obtaining optimal outcome.Our study shows that decreased hair density in FPHL patients is associated with altered serum vitamin D and zinc level. Though other triggering factors like family history (19),altered androgen level(20),altered serum iron (21) may be associated with FPHL patients, a highly statistically significant difference between patients and control regarding mean serum level of 25- (OH) D and zinc proves this fact. Our study matched with Hoda Moneib et al. (22) and H. Rasheed et al. (23) who also showed significant decrease in serum 25-(OH) D levels in patients of FPHL as compared to controls. A statistical decrease in serum 25-(OH) D level with increase in severity or grading of FPHL as was found in our study matched with H. Rasheed et al. (23) whereas an increase in 25-(OH) D level with increase in severity was noted by Hoda Moneib et al.(22). Regarding serum zinc status in patients of FPHL our study matched with Min Seong Kil et al. (24) who also noticed a significant decrease in case group as compared to control.

Zinc is an essential micronutrient that is required for the function of more than 300 enzymes in our body (25).
Zinc is believed to play crucial role in DNA & RNA production and in cell division. So zinc becomes essential for normal division of hair follicle cell that leads to healthy hair growth (26).

Zinc has also been proved to possess antioxidant property, so it seems to delay aging phenomenon and so hair loss (23).

More extensive studies on humans are needed to establish the exact role of vitamin D in hair cycle. However topical application of vitamin D has been proved to be beneficial against chemotherapy induced alopecia in mouse model (27).Vitamin D has also been used in treatment of hair loss due to scalp psoriasis (28).Vitamin D has also been beneficial in radiation induced alopecia probably by upregulating VDR (vitamin D receptor) (29).

In our study we have found a significant decrease in serum vitamin D level with increase in severity or grading of FPHL patients. This means that if vitamin D deficiency/insufficiency is left unnoticed in milder form (gradeI), there will be further decrease in vitamin D level with increasing time thus leading to increase in severity or grade of FPHL.

Conclusion:
Our study suggest that all patients of FPHL must be screened for serum level of vitamin D and zinc so that early supplementation of these in FPHL patients may prove to be beneficial and may halt or at least delay the progress of the disease from milder to severe form. However large study are needed to es-

tablish the fact that whether vitamin D and zinc supplementation in susceptible individuals (ie.with family history) would prevent the appearance of the disease in them and also to establish that whether vitamin D and zinc supplementation in FPHL patients may revert the condition.

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