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A study of biochemical factors and knowledge, attitude, and practice in patients with premature graying of hair

Erken saç beyazlaması olan hastalarda biyokimyasal faktörler ve bilgi, tutum, uygulama çalışması

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Abstract

Background and Design: Premature graying of hair (PGH) is a clinical entity with multiple ramifications such as low self- esteem, inferiority complex and may interfere with an individual's social interaction capabilities and adversely affect the individual's psychosocial health. This study aimed to investigate the biochemical factors and knowledge, attitude, and practice in patients with PGH.

Materials and Methods: A case control study including 75 cases and equal number of age and sex matched controls was conducted in a tertiary care hospital. Various epidemiological variables and biochemical parameters (vitamin B_{12}, vitamin D, calcium, iron profile, lipid profile, thyroid hormones) were ascertained and compared between the cases and controls. The severity of graying was assessed using the Graying Severity Score, and a knowledge, attitude, and practice study was conducted by administering a pre-validated questionnaire to assess the impact on the quality of life of these affected individuals.

Results: Significant differences between cases and controls in the serum levels of biochemical parameters, namely Vitamin B_{12} (p=0.001), vitamin D (p=0.004), serum iron (p<0.001), ferritin (p<0.001) and total iron binding capacity, i.e TIBC (p=0.037) and thyroid hormones T4 (p=0.002) and thyroid stimulating hormone (p=0.041) was found. No correlation between PGH and serum calcium and lipid profile was derived. Also, a statistically significant difference was noted in the appetite pattern of cases and controls (p=0.003). Stress, smoking, altered sleep pattern and bowel habits were also more prevalent in cases as compared to controls. It was also noted that most study cases belonged to the lower middle class of the modified Kuppuswamy scale. The Knowledge, Attitudes, and Practices (KAP) study, which was extremely comprehensive and detailed in nature, showed that PGH significantly affects these patients' quality of life.

Conclusion: The study reveals a strong association between PGH and deficiency of vitamin D, abnormal levels of vitamin B_{12}, hypothyroidism and deranged iron profile, and altered appetite patterns. Along with this, the prevalence of stress, smoking, altered sleep patterns and bowel habits was also higher in PGH cases. Hence, all these parameters should be analysed in such patients. KAP analysis was a unique approach to studying common conceptions and practices in PGH cases and its' psychological impact.

Keywords: Premature, grey hair, biochemical parameters, case control study, KAP analysis

Öz

Amaç: Erken saç beyazlaması (ESB), düşük benlik saygısı, aşağılık kompleksi gibi birçok sonucu olan ve bireyin sosyal etkileşim yeteneklerini etkileyebilen ve dolayısıyla bireyin psikososyal sağlığını olumsuz yönde etkileyebilen klinik bir antitedir. Hindistan alt kıtasında ESB ile ilgili epidemiyolojik, araştırmacı ve psikanalitik çalışmaların azlığı nedeniyle, bu çalışma ESB olan hastalarda biyokimyasal faktörlerin rolünü belirlemek ve bilgi, tutum ve uygulamaları değerlendirmek için yapılmıştır.

Gereç ve Yöntem: Üçüncü basamak bir hastanede 75 olgu ve eşit sayıda yaş ve cinsiyet eşleştirilmiş kontrol içeren bir olgu kontrol çalışması yürütüldü. Çeşitli epidemiyolojik değişkenler ve biyokimyasal parametreler (vitamin B_{12}, vitamin D, kalsiyum, demir profili, lipit profili, tiroid hormonları) tespit edildi ve bunlar olgular ve kontroller arasında karşılaştırıldı. Grileşmenin şiddeti, Graying Şiddet Skoru kullanılarak değerlendirildi ve bunun etkilenen bireylerin yaşam kalitesi üzerindeki etkisini değerlendirmek için önceden doğrulanmış bir anket uygulanarak bir bilgi, tutum, uygulama çalışması yapıldı.

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Bulgular: Olgular ve kontroller arasında vitamin B_{12} (p=0,001), vitamin D (p=0,004), serum demiri (p<0,001), ferritin (p<0,001), total demir bağlama kapasitesi (p=0,037) ve tiroid hormonları T4 (p=0,002) ve tiroid uyarıcı hormon (p=0,041) düzeyleri açısından istatistiksel olarak anlamlı farklılık saptandı. ESB ile serum kalsiyum ve lipid profili arasında bir korelasyon elde edilmedi. Bununla birlikte, olgu ve kontrollerin iştah paterninde istatistiksel olarak anlamlı fark kaydedildi (p=0,003). Kontrollere kıyasla olgularda stres, sigara içme, değişen uyku düzeni ve bağırsak alışkanlıkları da daha yaygındı. Çalışma olgularının çoğunun değiştirilmiş Kuppuswamy ölçeğinin alt orta sınıfına ait olduğu da kaydedildi. Bilgi, Tutumlar ve Uygulamalar (KAP) çalışması ile ESB'nin bu hastaların yaşam kalitelerini önemli ölçüde etkilediği görüldü.
Sonuç: Çalışma, ESB ile vitamin D eksikliği, anormal vitamin B_{12} seviyeleri, hipotiroidizm ve dengesiz demir profili ve değişen iştah paternleri arasında güçlü bir ilişki olduğunu ortaya koyuyor. Bununla birlikte stres, sigara içme, değişen uyku düzeni ve bağırsak alışkanlıkları da ESB olgularında daha yüksekti. Bu nedenle bu tür hastalarda tüm bu parametrelerin analiz edilmesi gerekmektedir. KAP analizi, ESB olgularındaki ortak anlayış ve uygulamaları ve bunların psikolojik etkilerini incelemek için benzersiz bir yaklaşımdı.
Anahtar Kelimeler: Erken, gri saç, biyokimyasal parametreler, olgu kontrol çalışması, KAP analizi

Introduction

Premature graying of hair (PGH) is graying of hair that occurs before the age of 20 years in Whites, 25 years in Asians and 30 years in Africans[1]. It may occur alone or with the additional role of various acquired and environmental factors, such as smoking, stress, autoimmune disorders or nutritional deficiencies. Etiopathogenesis: One mechanism of pathogenesis of graying of hair is the reduction in melanogenically active melanocytes in the hair bulb of grey anagen hair follicles, resulting in pigment loss. Secondly, melanocyte degeneration leading to melanin incontinence and defective transfer of melanosomes to cortical keratinocytes also contribute to the hair graying process. In genetic disorders like progeroid syndromes, defects in the DNA repair system and oxidative stress are the major factors responsible for hair graying.

Role of biochemical factors: DNA synthesis is highly dependent on vitamin B_{12} and folic acid; hence sufficient supply of both vitamin B_{12} and folic acid are required for the proliferation of cells in the hair follicle. Calcium is involved in a few steps of the process of melanogenesis. Also, PGH has been linked to reduced bone mineral density. Evidence provided by studies suggest the role of iron in the modulation of the enzyme tyrosinase, an essential enzyme in melanogenesis. In their study, van Beek et al.[2] showed that triiodothyronine (T3) and tetraiodothyronine (T4) can significantly stimulate melanin synthesis in the hair follicle. Many researchers believe that premature graying might be a predictor of an underlying systemic disease, particularly cardiovascular disease. ElFaramawy et al.[3] suggested that atherosclerosis and hair graying have similar mechanisms of DNA repair impairment, oxidant stress, androgens, inflammatory process and incidence of both increases with age. Therefore, the serum lipid profile of individuals with PGH might be an essential parameter to be considered.

Role of smoking and stress: In a study conducted by Zayed et al.[4] it was concluded that smokers had an earlier onset of hair graying than non-smokers. Smoking, stress, and other factors like ultraviolet rays, pollution, emotional factors, or inflammatory causes lead to oxidative damage to the hair follicle melanocytes. It is hypothesized that prolonged stress may lead to oxidative cellular damage, reduced telomerase activity and shortened telomere length. A study by Epel et al.[5] showed that women with the highest levels of perceived stress have telomeres shorter on average, equivalent to an additional aging of at least one decade compared to women with low levels of perceived stress.

Quality of life: People with PGH may develop an inferiority complex due to discrimination by family and friends and rejections in relationships, which may affect their psychosocial health. As seen in the study of impact on quality of life in premature canities in 52 patients by Daulatabad et al.[6], PGH was found to have a massive impact on the quality of life of the affected individuals, causing mood fluctuations and a sense of guilt.

As there is a paucity of epidemiological, investigative and psychoanalytical studies on PGH from the Indian subcontinent, the present study has been undertaken to ascertain the role of biochemical factors and assess knowledge, attitude, and practice in patients of PGH to explore the possible association of this entity with common systemic disorders and deficiencies which may be related to its etiology and study comprehensively in detail the impact of quality of life in these patients of PGH by using a detailed Knowledge, Attitudes, and Practices (KAP) questionnaire.

Materials and Methods

A case-control study including 75 cases and an equal number of age and sex-matched controls in the age group 7-25 years was conducted in a tertiary care hospital from October 2019 to September 2021 after approval from ESI-Post Graduate Institute of Medical Sciences and Research Ethics Committee (Ethical number: ESIPGIMSR-IEC/2019007, date: 14.09.2019). Informed and written consent was taken from all study subjects (in the case of minors, the consent was given by parent(s)/guardian/caregiver along with oral assent for children between 7-11 years of age, and written assent for children between 12-18 years of age). Patients aged >25 years, or with graying of hair as a part of other conditions such as vitiligo, progeria, Werner's syndrome, Rothmund-Thomson syndrome and those who refuse to consent to undergo investigations were excluded from the study.

A predesigned proforma was completed with the particulars of the site(s) of involvement, duration of disease, personal, family history, treatment history & presence/absence of associated systemic disorders. Various epidemiological variables and biochemical parameters (vitamin B_{12}, vitamin D, calcium, iron profile, lipid profile, thyroid hormones) were recorded and compared among cases and controls.

The severity of graying was assessed using Graying Severity Score (GSS), proposed by Singal et al.[7]. GSS-the entire scalp surface was divided into 5 zones - the frontal region, vertex, right and left temporal regions, and the occipital region. In each of these zones, areas showing maximum graying were identified, and a 1 cm^2 area was marked in each region (Figures 1-5). Based on the hair count in these squares, scores according to the percentage of grey hair were recorded as follows:

- Score 1 (<10% grey hair/cm^2),
- Score 2 (10-30% grey hair/cm^2),
- Score 3 (>30% grey hair/cm^2).

The GSS was calculated by taking a sum of these scores at the five sites. The objective scores are further graded as:
- Mild (a score of 0-5),
- Moderate (score of 6-10),
- Severe (score of 11-15).

Knowledge, attitude, and practice study was conducted through a pre-validated set of 30 questions to assess impact on quality of life of the affected individuals.

Statistical analysis

Categorical variables are presented in number and percentage (%), and continuous variables are presented as mean ± standard deviation and median. The normality of data is tested by the Kolmogorov-Smirnov test. If the normality is rejected, then non-parametric test will be used. Statistical tests were applied as follows:

1. Quantitative variables are compared using the Unpaired t-test/Mann-Whitney U test (when the data sets were not normally distributed) between the two groups.
2. Qualitative variables are compared using chi-square test/Fisher's exact test. A p-value of <0.05 will be considered statistically significant. The data is entered into an MS excel spreadsheet, and analysis is done using SPSS version 21.0.

Figure 1. Shows cropped hair in the marked area for easier visualization and calculation of percentage of grey hair in the marked area

Figure 2. Shows right temporal region of scalp for GSS calculation
GSS: Graying Severity Score

Results

Demographic profile: The mean age of cases and controls was 18.44±4.303 years. The mean age at onset of PGH was 12.5±3.2 years, with the earliest onset reported at five years in an 8-year-old male patient. The majority of cases were in the 21-25 age group (40%). 73.3% were males, 26.6% were females. The majority were from the lower middle class (45.3%), followed by upper lower (18.7%) and upper middle class (17.3%). Approximately 9.3% were in each extreme group (upper and lower class).

Clinical profile: All cases showed scalp involvement. Among 44 males over 15 years, 5 had beard involvement, while sideburn involvement was observed in 8% (6 patients). Axilla/groin involvement was not observed in any case. The frontal area of the scalp was the site of origin in 56% of patients, followed by 18.7% in the temporal region, 24% in the vertex, and 13.3% in the occipital region. At the time of presentation, 48% (36 patients) had diffuse graying, 13.33% had single-site involvement, and 38.7% had 2-3 sites involved.

Out of 75 cases, 44 had no symptoms of biochemical abnormalities. Among the remaining 35 cases, 17 reported fatigue/dizziness/weakness, four reported tingling/numbness (vitamin B_{12} deficiency), 6 reported joint pains (vitamin D/calcium deficiency), and four reported symptoms related to thyroid abnormalities.

A statistically significant difference (p=0.003) was found in the appetite pattern between cases and controls. Out of 75 patients, 15 (20%) had altered appetite, while only 3 out of 75 controls had reduced appetite. Nearly all subjects with altered appetite showed abnormalities in their

Figure 3. Shows left temporal region of the scalp For GSS calculation
GSS: Graying Severity Score

Figure 4. Shows frontal region of scalp for GSS calculation
GSS: Graying Severity Score

biochemical parameters, including low vitamin D levels in 93.3% of patients and 33.3% of controls, reduced calcium levels in 80% of patients and 33.3% of controls, deranged iron profile in 46.7% of patients, deranged thyroid profile in 33.3% of patients, and abnormal vitamin B_{12} levels in 26.7% of patients and 33.3% of controls. Altered sleep patterns were reported in 7 cases and 2 controls, with a p-value of 0.166. Additionally, altered bowel habits (constipation) were reported in 13.3% of cases and 5.3% of controls, with no reported bladder abnormalities. The two groups had no significant difference in dietary habits (p=0.191). Moreover, 12% of cases reported occasional smoking compared to 4% of controls (p=0.130). Additionally, 20% of patients reported mood disturbances such as stress, anxiety, or depression. Furthermore, most of the cases reported using non-medicated shampoos (68%), oiling their hair (97.3%), and using hair dye (60%).

Of 75 patients, 20% had a positive family history of premature grey hair, primarily affecting first-degree relatives. Regarding graying severity, 54.7% fell into the mild category, 41.3% fell into the moderate category, and only 4% had severe graying. The average GSS was 5.6±2.48.

Investigations: Data for laboratory values for cases and controls is listed in Table 1.

Knowledge, Attitude, Practice Assessment

In our study, we observed that 68% of cases reported having little to no knowledge about the potential causes of PGH. Some 69.3% of cases did not consider PGH a contagious condition, while 8% did consider it communicable. Regarding the perception of inheritance, 9.3% of cases thought there are "very much" chances (50-100%) of their children inheriting PGH from them, 10.7% of cases thought there are 25-50% chances of their children getting this condition, while 38.7% thought there is a low probability (<25%) of their children inheriting this condition. 41.3% of cases (31 patients) thought there was almost no chance of their children getting PGH because of them. The majority of cases (36%) thought there was no association of lack of self-care with their PGH. Some 22.7% of cases considered there are "little" chance of lack of self-care as the reason for their PGH, and only 12% considered lack of self-care as the very likely cause of their condition. Approximately 29.3% of cases considered 25-50% chances of this association.

Figure 5. Shows occipital region of scalp for GSS calculation
GSS: Graying Severity Score

It was observed that 16% of cases felt diet has a very significant role (50-100%) in PGH, and 24% felt diet has a 25-50% role in graying of hair. Some 33.3% (25 cases) thought diet has "little" role in PGH, while the rest 26.7% thought there is no role of diet in this condition. Furthermore, 33.3% and 9.3% of cases believed that dandruff was the root cause of PGH, with 50-100% chances and 25-100% chances, respectively. In contrast, 20% of cases thought there was no association between dandruff and PGH, while the remaining 37.3% believed there were "little" chances (<25%) of a relationship between dandruff and PGH. Regarding the potential role of previous illnesses in PGH, the majority of cases (42.7%) considered 'little' (<25% chances) of their previous illness being the cause of their PGH, as most of them did not have a history of chronic medical illness. Meanwhile, 34.7% did not consider any relation with previous illness and PGH, while 17.3% considered 25-50% chances, and the remaining 5.3% of cases considered "a lot" chances (>50%) of this association. In terms of stress, 26 cases (34.7%) considered "little" (<25%) chances of stress being related to their PGH, 30.7% considered 25-50% chances, and 14.7% considered "very much" (>50%) chances of stress being the cause of their PGH. Fifteen patients (20%) did not relate stress to PGH. Some 57.3% of cases considered high chances (>25%) of PGH being just a physiological change, adversely affecting their looks. On the other hand, 33.3% thought it had a "little" effect on their appearance, while 9.3% did not think it made any difference. Additionally, 80% of cases did not believe they are aging faster due to their PGH, 16% thought they might be aging a "little" faster (<25% chances), and 4% strongly felt that they are aging faster because of PGH. Furthermore, 8% of cases felt "very much" self-conscious or embarrassed due to PGH, while 22.7% felt "a lot" self-conscious. In contrast, 37.3% of patients felt "a little" embarrassed because of PGH, and 32% did not feel any embarrassment due to their PGH. Lastly, 6 cases (8%) felt that PGH hampers their interaction with others very much, 24% thought it hampers it a lot, 37.3% believed it has "little" effect on their social interaction, and 30.7% felt that PGH does not hinder their interaction at all.

Regarding the perceived impact of PGH on appearance, 76% of cases believed it made them look much older than their actual age, while 18.7% considered it made them appear older "a little." Only 5.3% did not think PGH made them appear older than their actual age. Regarding the impact of PGH on social life, 30.7% of cases did not feel hesitant in attending social gatherings, 32% felt "a little" hesitant, and 29.3% felt "a lot" or "very much" hesitant. Concerning job interviews, 2 cases faced rejection "a little", and 1 case faced it "a lot." This question was applicable to only 14 out of the total 75 cases. In terms of personal relationships, 7 cases (38.8% of 18 patients) reported facing rejection "a few" times, while 4 of 18 patients (22.2%) faced it "a lot." This question was applicable to only 18 out of the total 75 cases.

53.3% reported being pointed out 'very much' for their PGH by friends/relatives/partner/parents. Some 17.3% reported being pointed out "a lot." While 13.3% reported such events a few times, 16% reported that they have never been singled out for their PGH by friends/ relatives/parents/partner. The majority (62.7%) reported no arguments related to PGH with their friends/relatives/parents/

partner, 8% reported unwanted arguments a few times, while remaining (18.7% - "a lot" +10.7%- "very often") reported such events a lot often. Two cases reported "a little" difficulty concentrating in work because of PGH, 11 did not feel any difficulty ("not at all") concentrating in their work because of PGH. The question was applicable to only 13 out of 75 cases. The majority of cases (69.3%) did not feel any difficulty concentrating in studies due to PGH, 20% felt "a little" difficulty, 9.3% faced "a lot" difficulty in concentration while only 1 case (1.3%) reported "very much" concentration difficulty in studies owing to PGH. Twenty-five (78.1% of 32 cases) did not feel that mental stress due to PGH has affected their libido/sexual performance; rest 7 cases felt it might have affected their libido/sexual performance "a little." The question was relevant/applicable to only 32 out of 75 cases. Sixteen percent of the cases did not feel any need for taking treatment, 38.7 % felt "little" need for taking treatment while "a lot" and "very much" need were felt among 29.3% and 16% cases respectively. Of 57 cases who reported using home remedies, the majority of cases reported no improvement (59.6% - "not at all"), while 31.5% reported only "little" improvement, 8.7% reported "a lot" improvement with home remedies. Eighteen cases reported not using any home remedies. Some 41.3% cases reported putting "little" effort for concealing their grey hair at home (dyes/camouflage etc.), while 53.3% (36% - "a lot" + 17.3% - "very much") reported putting significant efforts into concealing their grey hair at home under the influence of advertisements (television/print media), while 4 cases (5.3%) reported not using this method. 16.6% (8 out of 48 cases who have used the above methods) reported very much success in concealing their grey hair at home, while 16.6% of the 48 cases reported 25-50% success, 41.6% reported this to be only "a little" helpful, while 25% (12 out of 48 cases) reported this to be of no help in concealing their grey hair ("not at all"). A large percentage (89.3%) did not report experiencing so much anxiety and depression that they ever needed the help of a medical counsellor, while 8 cases (10.7%) felt this need only "a little." Some 78.7% of cases did not report taking medical opinion from a trichologist/dermatologist for this condition, 14 cases (18.7%) reported seeking medical advice a few times, while 2 cases (2.7%) reported seeking advice for their condition "a lot" of times. While 12 cases (16%) thought medications improve this condition "very much", 29 cases (38.7%) felt it can provide 25-50% improvement, 40% thought medications will provide only "little" improvement. Four of them (5.3%) thought medications will not show any improvement in this condition. Three cases (4%) felt "very much" burdened financially to take care of their PGH, 12% felt "a lot" burdened, 34.7% felt "little" burdened, and 49.3% (37 patients) did not feel any financial burden related to this condition.

Discussion

The demographic profile of our study population is generally consistent with previous research, although there are some slight variations. Specifically, the mean age of both cases and controls in our study was slightly higher than the mean age reported in the study by Sharma and Dogra[8], which was 15.71 years. Similarly, the mean age at onset of PGH in our study was slightly higher than the mean age reported by Sonthalia et al.[9], which was 10.2±3.6 years. The earliest age of onset of PGH reported was the same as Sonthalia et al.[9], however, the age of onset of 3 years has also been reported by Daulatabad et al.[10]. The majority of study cases belonged to the age group 21-25 years (40%), while in the study by Sharma and Dogra[8], most of the subjects belonged to the 11-15 years age group (34.17%). Although all of these studies reported a higher number of male cases, the numbers of male participants reported in the present study were much higher compared to data reported by Sharma and Dogra[8], Sonthalia et al.[9], and Daulatabad et al.[10].

Table 1. Data for laboratory values for cases and controls

Laboratory parameters	Cases		Controls		p-values
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
Vitamin B_{12}	362.2±325.7	206.7 (257.05)	380.0±214.6	348.0 (328.2)	0.001
Vitamin D	24±13.5	19.8 (19.35)	31.1±14.3	30.4 (18.7)	0.004
Serum calcium	8.8±0.89	9.0 (1.2)	8.8±0.89	9.0 (0.9)	0.099
Serum iron	92.5±66.7	80.0 (98)	108.8±51.8	100.2 (61.6)	<0.001
TIBC	354.3±86	366.0 (154.95)	368.6±104.8	352.0 (118.9)	0.037
Serum ferritin	124.8±170.5	82.05 (107.275)	103.8±66.8	97.0 (92.1)	<0.001
T. chol	167.8±35.1	168.0 (49)	160.1±29.5	159.0 (46.0)	0.071
LDL	100.30±32.2	97.8 (37)	91.3±25.3	90.4 (39.3)	0.184
VLDL	26.1±11.5	24.6 (16.2)	25.7±7.4	23.0 (8.9)	0.666
TG	128±52.9	123.0 (79)	128.4±37.2	115.0 (44.5)	0.827
HDL	41.3±9.7	42.0 (13)	43.2±6.8	44.0 (5.0)	1.000
Triiodothyronine (T3)	3±0.8	3.0 (1.45)	3.1±0.9	3.0 (1.6)	1.000
Thyroxine (T4)	1.1±0.5	1.1 (0.6)	1.3±0.4	1.2 (0.7)	0.002
TSH	4.1±6.3	2.4 (2.9)	3.4±3.7	3.0 (2.0)	0.041

P-values for each parameter are calculated using Mann-Whitney U test (non-parametric test). SD: Standard deviation, IQR: Interquartile range, TIBC: Total iron binding capacity, T. chol.: Total cholesterol, LDL: Low-density lipoprotein, VLDL: Very-low-density lipoprotein, TG: Triglyceride, HDL: High-density lipoprotein, TSH: Thyroid-stimulating hormone

The majority of cases in the study belonged to the lower middle class. Socio-economic status may play a significant role in nutrition, knowledge and attitude of the patients. To our knowledge, no studies correlating socioeconomic status with premature grey hair have been conducted to date. However, in the present study, the cases and controls enrolled were solely the beneficiaries of this specific hospital; hence, the possibility of selection bias cannot be ruled out.

It was observed that scalp involvement was universal, with all cases showing graying of the scalp. This aligns with existing literature and confirms premature graying primarily affects the scalp. Interestingly, involvement of the beard was in 6.7% of cases and sideburns were also noted in 8% of cases. This finding suggests that premature graying may extend beyond the scalp to other hairy areas in some individuals. This contrasts a study by Daulatabad et al.[10], which found no involvement of non-scalp areas. The variation in involvement of different hair areas could be attributed to genetic predisposition or hormonal influences, or regional differences in melanocyte activity, for which further research is needed to explore the underlying mechanisms.

Regarding the specific sites of scalp involvement, we found that the frontal area was the most commonly affected, accounting for 56% of cases. This finding is consistent with the study by Daulatabad et al.[10], which reported a similar prevalence of frontal involvement. However, other studies have reported different patterns. Sharma and Dogra[8] found that the vertex was the first site to be involved in most cases, followed by the frontal area. In contrast, Sonthalia et al.[9] reported that the temporal region was the most commonly affected site.

Most cases in our study presented diffuse graying of the scalp, which is consistent with findings from previous studies by Sharma and Dogra[8] and Daulatabad et al.[10]. However, we also noted that 13.33% of cases had involvement of a single site. Sharma and Dogra[8] reported a slightly lower percentage (5%) of cases with single-site involvement.

On being asked, 35 cases reported symptoms of nutritional deficiencies or thyroid abnormalities. It is important to note that these symptoms are not specific to a particular biochemical abnormality, and further laboratory investigations are required to confirm the suspected abnormalities. Upon further testing, most of these patients were found to have confirmed biochemical abnormalities. Patients of Sonthalia et al.[9] reported no specific symptoms of biochemical abnormality, only c/o general feeling of weakness during physical activity was reported by these patients.

A significant difference (p-value=0.003) between appetite pattern of cases and controls was noted. Altered appetite habits leading to nutritional deficiencies may have contributed to the premature graying process in these cases, as reflected in their laboratory investigations. Chakrabarty et al.[11] also observed irregular eating habits in a significant proportion of patients (p<0.001).

Regarding sleep patterns, no significant results were obtained in this study. However, disturbed sleep can affect general well-being of a person, and dietary habits might cause stress as well, which can work as a contributing factor in the PGH of an individual, therefore, further research is required to explore the relationship of sleep habits with PGH. There was no significant difference in bowel and bladder habits between cases and controls. The subjective nature of these complaints and the potential relationship between reduced appetite and reduced frequency of bowel movements may explain the lack of statistical significance in our findings. Dietary habits, especially the consumption of non-vegetarian food as a source of vital nutrients like vitamin B_{12}, were important factors in an individual's nutrition. In our study, many vegetarian cases showed vitamin B_{12} deficiency compared to those who consumed a mixed diet. This highlights the importance of dietary habits and the potential impact on nutrient deficiencies, specifically vitamin B_{12}. However, statistically, no significant difference in nutritional habits was observed between cases and controls.

Our study did not find a statistically significant difference in smoking habits between cases and controls. However, other studies have reported significant associations between smoking and premature graying. For example, Sharma and Dogra[8] found that 11.6% of cases were smokers compared to only 0.83% of controls. Similarly, Sabharwal et al.[12] also demonstrated a significant association between tobacco use and hair graying. The oxidative stress due to the huge amount of reactive oxygen species generated due to smoking could damage the melanocytes. Hence, the melanocytes of grey hair bulbs are highly vacuolated, owing to the increased oxidative stress.

Regarding stress, we found that 20% of cases reported experiencing mood disturbances. Additionally, in the KAP study conducted among the cases, 14.7% of patients considered stress to be a very likely reason for their premature graying. This finding is consistent with the study by Sharma and Dogra[8], who used the Perceived Stress Severity (PSS) scoring system and reported significantly higher PSS scores in 83.59% of their cases. It is well-established that stress can cause oxidative damage to melanocytes, leading to premature graying, and conversely, premature graying can also contribute to stress in individuals.

Our study found that most cases reported regular attention to their hair and scalp hygiene, including frequent shampooing and oiling. The use of non-medicated shampoos was common among cases, which could be attributed to the fact that most patients did not seek medical opinion for their premature graying. Interestingly, we did not observe any apparent beneficial effect of hair oils in our cases, which contrasts the findings of Sharma and Dogra[8], who reported a protective role of hair oiling in their study. Regarding the use of hair dye, we found that 60% of cases reported using hair dye. This is consistent with the responses from the KAP survey, where a significant proportion of patients reported putting substantial efforts into concealing their grey hair using hair dyes. However, most patients considered the helpfulness of hair dyes to be only minimal, likely due to the temporary nature of the results as the dye color eventually fades away.

In our study, 20% of patients reported a positive family history of premature graying. This finding is consistent with the studies by Sharma and Dogra[8], Sonthalia et al.[9] and Daulatabad et al.[10], who reported positive family histories in 90.1%, 65.83%, and 75% of patients, respectively. It is worth noting that the percentage of positive family history reported in our study is lower compared to these studies.

Regarding the GSS, we found that most cases (54.7%) fell into the mild GSS group, followed by 41.3% in the moderate GSS group. Severe GSS was seen in only 4% of cases. The mean GSS in our study was 5.6±2.48. This differs from the findings of Singal et al.[7], who observed that most patients fell into the moderate GSS category (61.54%), followed by 32.69% with mild GSS and only 5.77% with severe GSS. The mean GSS in their study was 6.6±1.97. However, both studies reported the same highest GSS score of 13.

Our study did not find statistically significant differences in lipid profile parameters between cases and controls. However, it is worth noting that total cholesterol values were abnormal in a higher percentage of cases (16.7%) compared to controls (6.7%), although the p-value did not reach statistical significance (p=0.07). Similarly, no significant differences were observed for triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein levels. This is in contrast to findings by Sharma and Dogra[8] and by Chakrabarty et al.[11] where statistically significant differences were noted for serum LDL (p=0.01) and HDL (p=0.03) in former and for HDL (p<0.001) in the latter.

Calcium is said to play a role in the steps of melanogenesis and, hence, can be an important contributing factor in PGH. Lower serum calcium was noted in 33.3 % (25 cases) compared to 16 controls (21.3%), but p-value was 0.099. However, statistically significant results were observed by Sharma and Dogra[8] and Bhat et al.[13], where p-values were (p<0.001) and (p=0.018) respectively.

PGH has been linked to reduced bone mineral density. Lower vitamin D levels were observed in 50 cases (70.4%) compared to only 34 controls (46.6%), and the p-value was 0.004. The results observed are in accordance with the results of Bhat et al.[13] who also reported significant lower levels of vitamin D levels in cases as compared to controls. However, Sharma and Dogra[8] and Chakrabarty et al.[11] who also observed lower levels of vitamin D in cases as compared to controls; reported that results were not statistically significant.

In addition to vitamin D, we also found abnormal vitamin B_{12} levels in a significant proportion of PGH cases compared to controls. This result aligns with the findings of Sharma and Dogra[8], Chakrabarty et al.[11], and Sonthalia et al.[9], who also reported significant associations between vitamin B_{12} deficiency and PGH. Hence, vitamin B_{12} is found to be significantly associated with prematurely grey hair as it plays a significant role in DNA synthesis of actively dividing cells of hair follicles. Also, statistically significant differences between cases and controls were noted in thyroid hormone levels, specifically T4 and thyroid stimulating hormone (TSH). Abnormal T4 levels were observed in a higher percentage of cases compared to controls, indicating a potential association between hypothyroidism and PGH. This finding is consistent with the study by Sonthalia et al.[9], who also reported significantly higher TSH levels in cases compared to controls. However, it is interesting that the association between PGH and thyroid abnormalities is not universally consistent across studies. Daulatabad et al.[10] found no significant association between PGH and thyroid abnormalities. This suggests that the relationship between PGH and thyroid dysfunction may be complex and influenced by various factors.

Regarding iron profile, we found significant differences in serum iron, ferritin, and total iron binding capacity (TIBC) levels between cases and controls. Lower serum iron levels and abnormal ferritin levels were more common in cases compared to controls. These findings are consistent with studies by Sharma and Dogra[8], Bhat et al.[13], and Chakrabarty et al.[11], who also reported abnormal ferritin levels in PGH patients. No studies to compare serum iron levels and TIBC in PGH patients are available to our knowledge. The association between PGH and iron profile abnormalities may be explained by the role of iron in melanin production and hair pigmentation. Iron deficiency can disrupt melanin synthesis, leading to PGH. However, it is important to note that the results of serum ferritin levels reported by Sonthalia et al.[9] were statistically insignificant, indicating some variability in findings across studies.

KAP analysis suggests that PGH also tends to have a great psychosocial impact on an individual, with hair being a crucial part of physical appearance. The majority of study patients practised adequate local hair care, yet importance of diet was known in only 40%. Myths like the condition being contagious have also been noted among 8% of patients. The majority of patients reported that PGH is affecting their physical appearance, and they are being pointed out frequently for their condition, implying the psychological effects of the condition on an individual's life. A significant number of patients have reported PGH as the reason for unwanted arguments with their near and dear ones. Some teenagers (10.6% cases) reported losing concentration in studies because of the condition. Despite the majority feeling the need for treatment, only a few cases reported seeking professional help. Others reported using multiple forms of treatment based on advertisements/advice from friends/family. In order to get rid of PGH and to improve their physical appearance, individuals tend to buy expensive products, claiming to give immediate and successful results under the influence of advertisements, landing themselves into unwanted expenses. Thus, PGH can greatly affect an individual's outlook, approach and behavioural pattern, especially due to their young age group. The above observations are similar to what was observed by Daulatabad et al.[6], where the impact of PGH on quality of life was studied.

Study Limitations

Although the study was extensive in terms of detailed history taking, laboratory investigations, assessment of graying severity, and KAP analysis,the sample size was small due to the restricted number of research subjects available in the covid times. There may have been response biases, recall errors, or subjective interpretation. Time constraints have also limited the data collection.

Conclusion

The study reveals that PGH can start at a very early age, most commonly starting from the frontal and temporal areas, and multiple factors are attributed to the process of PGH, including various vitamins and minerals like vitamin $B_{12,}$ vitamin D, iron, calcium, lipids, hormonal factors like thyroid hormones, personal habits like smoking, dietary habits (like regular/irregular eating, type of diet consumed), stress, and genetics. A strong association was found between PGH and deficiency of vitamin D, abnormal levels of vitamin B_{12}, hypothyroidism and deranged iron profile. Also, statistically significant differences were noted in the appetite pattern of cases and controls. Serum calcium levels were also lower in cases than in controls (p=0.099). Stress, smoking, altered sleep patterns and bowel habits were also more prevalent in cases as compared to controls. Also, socioeconomic status was assessed for the first time in PGH patients in our study, where it was found that majority of cases (45.3%) belonged to the lower middle class of the modified Kuppuswamy scale, which might play a role in the nutrition, knowledge, attitude and practice of the patients. KAP analysis was also a unique attempt to study common myths, conceptions and practices among PGH patients. The detailed design of the questionnaire is also helpful

for an individualized approach to each case in terms of counselling and management, as a mere representation of the percentage of people affected mentally by the condition cannot define the impact it causes in a particular individual's life. Hence, there is a need to develop effective treatment modalities for those people who are in a vulnerable age group and are losing their self-confidence to this very benign yet psychologically demanding condition.

Ethics

Ethics Committee Approval: The approval of the ESI-Post Graduate Institute of Medical Sciences and Research Ethics Committee was received (approval number: ESIPGIMSR-IEC/2019007, date: 14.09.2019).

Informed Consent: Informed and written consent was taken from all study subjects.

Authorship Contributions

Surgical and Medical Practices: K.D., S.C., S.A., P.D., I.B., Concept: K.D., S.C., S.A., P.D., I.B., Design: K.D., S.C., S.A., P.D., I.B., Data Collection or Processing: K.D., S.C., S.A., P.D., I.B., Analysis or Interpretation: K.D., S.C., S.A., P.D., I.B., Literature Search: K.D., S.C., S.A., P.D., I.B., Writing: K.D., S.C., S.A., P.D., I.B.

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