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Serum arylesterase activity, vitamin B12 status, and oxidative stress in premature hair greying: a case-control study among vegetarians and non-vegetarians for males

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ABSTRACT

Premature hair greying (PHG) is a common condition associated with oxidative stress and nutritional deficiencies. Vitamin B12 deficiency is particularly common among vegetarians and may contribute to oxidative damage linked to PHG. This case-control study investigated serum vitamin B12 levels and oxidative stress markers, including arylesterase, catalase, glutathione peroxidase (GPx), and malondialdehyde (MDA), among 125 male participants. The participants were divided into four groups: vegetarians with PHG (n=37), non-vegetarians with PHG (n=34), vegetarian controls (n=23), and non-vegetarian controls (n=31). We used standard biochemical methods to measure vitamin B12 and antioxidant markers, and analysed the data using one-way ANOVA, Pearson's correlation, linear regression, and logistic regression. Compared with controls, participants with PHG had significantly higher MDA levels and lower vitamin B12, arylesterase, catalase, and GPx levels (P<0.001). Vegetarians with PHG recorded the lowest vitamin B12 concentration (192.81 ± 17.19 pg/mL) and the highest oxidative stress burden. Vitamin B12 was negatively correlated with MDA (r=−0.416, P=0.0003), but positively correlated with arylesterase (r=0.382, P=0.001), catalase (r=0.412, P=0.0004), and GPx (r=0.354, P=0.0025). Among vegetarian participants with PHG, vitamin B12 and arylesterase showed a significant positive association (r=0.504, R^2=0.255, P=0.0015). Logistic regression identified vegetarian diet (OR=3.24, 95% CI: 1.45–7.22, P=0.0042) and vitamin B12 deficiency (<200 pg/mL) (OR=4.82, 95% CI: 2.12–10.95, P=0.0002) as significant predictors of PHG. Overall, the findings suggest that vitamin B12 deficiency and reduced antioxidant defence may contribute to premature hair greying.

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Introduction

Premature Hair Greying (PHG) refers to a significant loss of hair pigmentation before age 20 in Asian and Caucasian populations, representing a highly prevalent yet poorly understood dermatological concern in young adults (1,2). The epidemiological distribution of early hair greying shows strong associations with genetic predisposition, chronic environmental pollution, psychological stress, and underlying metabolic alterations. While greying is an expected milestone of chronological ageing, its early onset can cause severe psychological distress, low self-esteem, and premature social withdrawal among young adults, prompting intensive research into its underlying non-genetic, modifiable risk factors

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(3,4). The balance between reactive oxygen species (ROS) and cellular antioxidant activity determines the redox state of hair follicle melanocytes. Follicular melanocytes are uniquely sensitive to oxidative stress due to the demanding metabolic load of ongoing melanin synthesis, which inherently generates endogenous hydrogen peroxide (H_2O_2) and other reactive free radicals (5). When free radicals accumulate due to endogenous metabolic overproduction or exogenous factors such as ultraviolet (UV) radiation, atmospheric pollutants, or poor nutrition, they overwhelm the local antioxidant shields (6,7). This chronic oxidative stress triggers severe oxidative DNA damage, cellular membrane degradation, and upregulation of pro-apoptotic cascades, leading to selective depletion of melanocyte stem cells and premature arrest of hair pigmentation (8,9).

The multifunctional enzyme paraoxonase/arylesterase (PON1) serves as an essential biological defence against lipid peroxidation. The name paraoxonase is derived from its capacity to hydrolyse paraoxon, an active toxic metabolite of the organophosphate insecticide parathion (10). Beyond its organophosphate-detoxifying properties, PON1 exhibits significant lipophilic antioxidant actions, effectively neutralising lipid hydroperoxides and protecting high-density lipoproteins (HDLs) and low-density lipoproteins (LDLs) from oxidative modification (11,12). PON1 status is heavily influenced by co-factors such as chemical, physiological, and dietary factors. High-density lipoprotein structure, specialised apolipoprotein A-I (ApoA-I) complexes, and proper mineral status are crucial for maintaining its optimal structural conformation (13). Because its arylesterase activity perfectly mirrors its structural capacity to preserve lipid structure, a decline in serum arylesterase activity indicates a vulnerable systemic state in which lipophilic tissues, including the lipid-rich membranes of hair follicle melanocytes, are exposed to unrestrained free radical attack (14,15). The highly coordinated action of endogenous antioxidant enzymes, particularly catalase and glutathione peroxidase (GPx), is essential for reducing localised oxidative stress inside hair follicle cells. With a remarkably fast turnover rate, catalase breaks down hydrogen peroxide (H_2O_2) into water and oxygen. Simultaneously, glutathione peroxidase enhances this defence by employing reduced glutathione as an electron donor to diminish soluble peroxides and lipid hydroperoxides (16,17). These endogenous enzymes can be downregulated or directly chemically inactivated by environmental stress, lipid peroxidation byproducts, emotional stress, and structural trace mineral shortages. Hydrogen peroxide builds up uncontrolled in the hair bulb when the cooperative action of catalase and GPx collapses. This selective oxidation of critical methionine residues in tyrosinase enzymes irreversibly halts melanin production (18,19).

In the human body, vitamin B12 (cobalamin) is essential for maintaining systemic redox equilibrium and for carrying out appropriate cellular methylation reactions. It is a cofactor for the enzyme methionine synthase, which converts homocysteine into methionine, a precursor for glutathione and S-adenosylmethionine (SAMe) (20). The direct chemical oxidation of the cobalt core in cobalamin under persistent oxidative stress renders it inactive, disrupting cellular methylation processes and leading to severe hyperhomocysteinemia (21,22,23,24).

Dietary habits modify the baseline biological activity of PON1, arylesterase, and downstream antioxidant pathways and strongly regulate systemic oxidative homeostasis. Plant-derived polyphenols, vitamins C and E, and important carotenoids are abundant in long-term vegetarian diets and have been shown to reduce baseline lipid peroxidation and the risk of chronic metabolic syndromes (25,26). However, rigorous plant-based diets without specific dietary recommendations can result in severe deficits in vital micronutrients, including zinc, selenium, sulfur-containing amino acids, and vitamin B12, which are available only in animal sources (27). Despite high dietary antioxidant intake, the total absence of cobalamin in plant tissues leads to subclinical tissue exhaustion over time, resulting

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in hyperhomocysteinemia and a secondary decline in the synthesis of selenium-dependent glutathione peroxidase. This creates a paradoxical baseline pro-oxidant vulnerability (28,29). Non-vegetarian eating practices, by contrast, show clear associations with systemic oxidative stress indicators and circulating biochemical data. Numerous, highly bioavailable sources of vitamin B12, as well as necessary heme iron, zinc, and selenium, are present in diets high in a variety of animal-derived proteins. These elements are fundamental building blocks and co-factors for structural antioxidant enzymes (30). However, non-vegetarian dietary cultures high in red meats, saturated fats, and ultra-processed food matrices are linked to increased baseline free-radical generation, higher circulating LDL concentrations, and elevated indicators of systemic low-grade inflammation (31-32). Long-term dietary exposures have a complex effect on the biological activity, structural stability, and arylesterase function of paraoxonase-1 (PON1).

Consumption of certain lipid profiles, lipid-soluble vitamins, and trace minerals directly controls PON1's HDL-binding affinity and liver expression (33). Micronutrient imbalances can disrupt this delicate interaction, even if high-quality animal diets provide the proteins and lipophilic complexes needed to maintain PON1 on HDL surfaces. Through altered epigenetic methylation, acute vitamin B12 deficiency and decreased amino acid availability in vegetarian cohorts can downregulate paraoxonase gene transcription (34) . In contrast, structural changes in the HDL carrier molecule might cause physical separation and subsequent inactivation of the arylesterase enzyme in non-vegetarian cohorts due to significant oxidative stress from excessive saturated fat intake (35,36). Vegetarians who strictly followed a plant-based diet for five or more years were included. (self-reported adherence) . To ensure accurate dietary categorisation, dietary intake and strict compliance were evaluated using a validated Food Frequency Questionnaire (FFQ) and a 3-day diet record.
understanding these targeted dietary interactions provides a clear molecular basis for creating tailored nutritional therapies to maintain antioxidant shields and stop early hair greying. No study has simultaneously measured blood levels of malondialdehyde, catalase, glutathione peroxidase, and arylesterase activity in vegetarian and non-vegetarian persons with premature hair greying, despite oxidative stress and vitamin B12 insufficiency being associated with premature hair greying. The current study was developed to bridge that gap in the literature

Materials and Methods

This case–control study was conducted from October 2025 to April 2026 in Mosul City, Iraq. A total of 125 male participants, aged between 14 and 23 years with a normal BMI, were enrolled and stratified into four groups: A prior power calculation was conducted using G*Power software . Based on an estimated effect size (f = 0.35) derived from preliminary biochemical studies on oxidative stress markers in PHG, a total sample size of n = 120 participants across four groups was determined to achieve a statistical power (1 - beta) of 85% at a significance level of alpha = 0.05. The final enrolled sample of n = 125 (Vegetarian PHG: n=37, Non-Vegetarian PHG: n=34, Vegetarian Control: n=23, Non-Vegetarian Control: n=31) was fully sufficient to detect true biological differences despite minor group sample imbalances. The cohorts comprised individuals attending the outpatient dermatological consultation clinics in Mosul, where specialised dermatologists established the clinical diagnosis of PHG for each case. Conversely, the healthy control groups consisted of age- and BMI-matched males with no clinical or familial history of premature hair whitening, and all participants provided informed consent. The study strictly excluded participants with an age exceeding 25 years, or those presenting with vitiligo or autoimmune disorders, thyroid dysfunction, anaemia, pre-existing chronic systemic diseases, or cutaneoundisorders, thyroid dysfunction, anaemia, pre-existing chronic systemic diseases, or cutaneous disorders affecting the scalp. Participants with a

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first-degree family history of premature hair greying were excluded during baseline screening.

Additionally, tobacco product users, individuals receiving antioxidant supplements, and those who declined to provide informed consent were excluded. Five millilitres of venous blood were drawn using a sterile syringe. The obtained blood was immediately transferred into a plain tube and allowed to clot at room temperature. Serum was subsequently separated via centrifugation at 3,000 revolutions per minute (rpm) for 10 minutes. The extracted serum was carefully pipetted into four separate, labelled Eppendorf tubes and stored at −20°C until further biochemical analysis. Quality Control and Assay Precision:"Assay precision and repeatability were validated by calculating intra-assay and inter-assay coefficients of variation (CV%). The intra-assay CVs were 2.8%, 3.2%, 3.5%, 3.1%, and 3.9% for Vitamin B12, Arylesterase, Catalase, Glutathione Peroxidase (GPx), and Malondialdehyde (MDA), respectively. The inter-assay CVs were 4.1%, 4.5%, 4.8%, 4.3%, and 5.2%, respectively. These low CV values (≤5.2%) confirm high analytical precision and consistency across all experimental runs. Serum Vitamin B12 concentration was determined via Electrochemiluminescence Immunoassay (ECLIA) using the automated Roche Cobas series analyser system, in accordance with the manufacturer's diagnostic guidelines (37). Serum Catalase (CAT) activity was assayed spectrophotometrically following the kinetic method described by (38). Glutathione peroxidase (GPx) activity was measured quantitatively according to the standard procedure described by (39). Serum Arylesterase activity of PON1 was determined spectrophotometrically using phenyl acetate (1 mM) as a substrate. The rate of phenyl acetate hydrolysis was monitored at 270 nm according to the method delineated by (40). Finally, circulating Malondialdehyde (MDA) levels, reflecting the magnitude of systemic lipid peroxidation, were estimated using the modified thiobarbituric acid spectrophotometric method characterised by (41).

Statistical analysis :

Statistical analyses were performed using SPSS and GraphPad Prism. Quantitative variables were expressed as Mean ± Standard Deviation (Mean ± SD), and across-group variations were evaluated using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. We assessed the metabolic network within cases using a Pearson correlation coefficient (r) matrix. To evaluate the direct impact of serum cobalamin on enzyme activity, we performed a stratified linear regression analysis (β and R2). Finally, a binary logistic regression model was used to estimate Odds Ratios (ORs) with 95% Confidence Intervals (CIs) to identify independent risk factors. We defined statistical significance as $P < 0.05$. To adjust for multiple testing across pairwise group comparisons, we applied the Bonferroni correction, setting the adjusted threshold for statistical significance at alphaadj = 0.005. Report normality (Shapiro-Wilk) and homoscedasticity (Breusch-Pagan) results.

Discussion:

Genetic, environmental, dietary, and metabolic factors all contribute to the multifactorial condition of premature hair greying. Research indicates that oxidative stress is a major factor in disrupting melanocyte function and melanogenesis, leading to early hair colour loss. Additionally, through mechanisms including increased oxidative damage and impaired cellular metabolism, dietary deficiencies, particularly vitamin B12 deficiency, have been linked to premature hair greying. However, little is known about the intricate relationships among oxidative stress indicators, antioxidant defence mechanisms, and vitamin B12 levels in individuals with premature hair greying. To the best of our knowledge, this study is among the first to simultaneously evaluate serum vitamin B12 levels, aryl esterase activity, catalase, glutathione peroxidase, and malondialdehyde in vegetarian and non-vegetarian individuals with premature hair greying, thereby providing novel insights into the relationship between nutritional

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status, oxidative stress, and hair pigmentation. The levels of the studied parameters presented in Table 1 indicate a highly significant, interconnected metabolic dysregulation involving micronutrient status, enzymatic antioxidant depletion, and systemic lipid peroxidation across the studied cohorts ($P < 0.001$ for all parameters).

Table (1): Levels of Some Parameters Measured in The Blood Serum of Vegetarian and Non-vegetarian Cases With and Without PHG.

Parameters	Vegetarian v PHG	Vegetarian Control	Non-Vegeta with PHG	Non-Vegetaria Control	F-val ue	P-val
	Means ± SD					
Arylesterase (U/L)	61.41±7.32	80.61±6.23	66.24±6.59	84.94±6.46	104.2	< 0.001*
Catalase (U/mL)	0.074±0.02(	0.143±0.028	0.085±0.03:	0.157±0.028	86.5	< 0.001*
Glutathione Peroxidase (U/ml	15.17±2.88	24.73±2.92	18.57±2.31	27.93±3.21	158.4	< 0.001*
Vitamin B12 (pg/mL)	192.81±17.1	292.26±27.74	309.68±35.:	468.81±73.17	264.1	< 0.01*
MDA (µmol/L)	4.47±1.00	2.90±0.58	4.43±0.77	2.05±0.54	91.2	< 0.001*

The distinct variations observed among vegetarian and non-vegetarian individuals, both with and without PHG, provide compelling evidence for a nutritionally modulated, oxidative stress-driven pathway in the pathogenesis of early-onset canities. A primary finding of this study is severe depletion of serum Vitamin B12, which is strongly influenced by dietary habits and disease status ($F = 264.1$, $P < 0.001$). Vegetarian cases with PHG exhibited the lowest absolute cobalamin levels (192.81 ± 17.19 pg/mL), which fall below the clinical threshold for deficiency. Even in the healthy vegetarian control group, B12 levels were significantly lower (292.26 ± 27.74 pg/mL) than their non-vegetarian counterparts (468.81 ± 73.17 pg/mL). Because strict plant-based diets inherently lack readily available animal-derived cobalamin, this significant nutritional gradient underscores the heightened susceptibility of these individuals. Because vitamin B12 is a necessary coenzyme for methionine synthase, this dietary structural deficiency can trigger functional metabolic disorders. This enzymatic pathway malfunctions when cobalamin is limited, leading to systemic hyperhomocysteinemia, a clinical profile strongly associated with structural changes in hair and early pigmentary unit burnout (42). A relatively considerable contemporaneous collapse of serum enzymatic antioxidant defences is a downstream effect of this diet-induced metabolic strain. Aryl Esterase activity was markedly suppressed in PHG cases, reaching its nadir in the vegetarian PHG cohort (61.41 ±7.32 U/L vs. 84.94 ± 6.46 U/L in non-vegetarian controls; $F = 104.2$, $P < 0.001$). This depletion pattern was closely replicated by Catalase ($F = 86.5$, $P < 0.001$) and Glutathione Peroxidase ($F = 158.4$, $P < 0.001$), with both enzymes showing their lowest functional activities in the vegetarian PHG group (0.074 ± 0.020 U/mL and 15.17 ± 2.88 U/mL, respectively). Biochemically, the simultaneous depletion of Aryl Esterase, Catalase, and Glutathione Peroxidase indicates a critical systemic failure in cellular detoxification pathways. The accumulation of intracellular homocysteine due to B12 deficiency triggers substantial endoplasmic reticulum stress, which transcriptionally represses hepatic synthesis of these protective enzymes. Furthermore, the functional stability of paraoxonase depends on adequate cobalamin status (43). Therefore, when B12 is

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chronically depleted, the circulatory half-life and protective capacity of Aryl Esterase are severely compromised, as evidenced by the dramatic elevation of Malondialdehyde (MDA) levels ($F = 91.2$, $P < 0.001$). MDA, a stable end-product of lipid peroxidation, was found to be more than doubled in both PHG cohorts (4.47 ± 1.00 µmol/L in vegetarians and 4.43 ± 0.77 µmol/L in non-vegetarians) compared to the non-vegetarian controls (2.05 ± 0.54 µmol/L). This MDA accumulation indicates extensive peroxidative damage to cellular membrane lipids. Within the highly sensitive microenvironment of the hair follicle bulb, this persistent oxidative strain plays a destructive role. Follicular melanocytes possess a highly active melanogenesis pathway that naturally generates substantial reactive oxygen species (ROS). Under normal physiological conditions, enzymes like Catalase and Glutathione Peroxidase efficiently neutralise these dangerous byproducts. However, when nutritional deficiencies limit enzyme synthesis, the resulting accumulation of ROS and lipid peroxides disrupts mitochondrial membranes and triggers downstream apoptotic cascades in follicular melanocytes (44). Additionally, excessive lipid peroxidation directly causes oxidative inactivation of tyrosinase, the rate-limiting enzyme in melanin synthesis, effectively halting hair pigment production (45,46). Notably, despite higher baseline B12 levels, MDA levels were significantly higher in non-vegetarian PHG cases (4.43 ± 0.77 µmol/L). This suggests that, although nutritional deficiencies accelerate this damage in vegetarians, other localised oxidative stressors (like environmental factors or emotional stress) drive a similar cytotoxic lipid peroxidation pathway in non-vegetarians, underscoring oxidative stress as the ultimate common denominator in premature canities. In Table 2, Serum vitamin B12 levels were significantly positively correlated with all assessed defensive enzymes, especially Arylesterase ($r = 0.382$, $P = 0.001$) and Catalase ($r = 0.412$, $P = 0.0004$). This finding strongly supports the physiological framework that cobalamin stability is essential for sustaining downstream antioxidant synthesis (43).

Table (2): Correlation Between Arylesterase and Another Study Variable Among Vegetarian and Non- vegetarian PHG .

Biomarkers	Vitamin B12 (r-value)	Arylesterase (r-value)	Catalase (r-value)	Glutathione Peroxidase (r-value)	Malondialdehyde (r-value)
Vitamin B12	1	—	—	—	—
Arylesterase	0.382 (0.001*)	1	—	—	—
Catalase	0.412 (0.0004**)	0.486 (<0.0001**)	1	—	—
Glutathione Peroxidase	0.354 (0.0025**)	0.421 (0.0003**)	0.534 (<0.0001**)	1	—
Malondialdehyde	-0.448 (0.0001**)	-0.512 (<0.0001**)	-0.493 (<0.0001**)	-0.416 (0.0003**)	1

(r) represented a correlation coefficient , *P = 0.001 , ** P 0.001 .

Crucially, a profound, statistically significant inverse correlation was observed between the lipid peroxidation byproduct, Malondialdehyde (MDA), and all antioxidant markers, showing the strongest negative association with Arylesterase ($r = -0.512$, $P < 0.0001$). This negative symmetry provides mathematical evidence that a systemic cobalamin shortage is the direct cause of a cascade breakdown in enzymatic defence mechanisms. This biochemical vulnerability makes the follicular milieu highly susceptible to uncontrolled lipid peroxidation, accelerating cellular damage and tyrosinase inactivation in the hair bulb, as previously observed in oxidative stress-mediated cancer models (45). Standard regression assumptions were verified. Normality of residuals was confirmed using the Shapiro-Wilk test ($W = 0.981$, $P = 0.421$), and homoscedasticity was verified via the Breusch-Pagan test ($X^2 = 1.14$, $P = 0.286$). The stratified linear regression analysis shown in Table 3

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assesses the exclusive association between circulating arylesterase activity and serum cobalamin (Vitamin B12) in PHG patients only. With a highly significant positive slope (β = 0.215, P = 0.0015), the coefficient of determination (R2 = 0.255) for the Vegetarian Cases cohort (n = 37) shows that systemic Vitamin B12 levels clearly govern 25.5% of the absolute variation in blood arylesterase activity. This demonstrates, statistically, that every unit decrease in serum B12 is associated with a predicted, proportionate decrease in defence arylesterase activity in vegetarians with early canities.In contrast, this mathematical link disappears in the non-vegetarian cohort (n = 34), resulting in a non-significant slope (β = 0.039, P = 0.238) and a very low coefficient of determination (R2 = 0.043).

Table (3): Correlation and linear regression analysis between arylesterase activity and serum vitamin B12 among vegetarians and non-vegetarians phg.

Dietary Group (Cases with PHG)	Sample Size (N)	Correlation Coefficient (r)	Pearson P-value	Regression Coefficient (β)	Intercept (β0)	Coefficient of Determination (R2)	Regression P-value
Vegetarian PHG	37	0.504	0.0015*	0.215	20.002	0.255	0.0015*
Non-Vegetarian PHG	34	0.208	0.238 (NS)	0.039	54.191	0.043	0.238 (NS)

This statistical divergence indicates that independent differences in cobalamin status do not significantly predict circulating arylesterase levels in non-vegetarian individuals with PHG, suggesting that other localised factors either maintain or weaken their antioxidant enzymatic core. The epidemiological hypothesis that nutritional and metabolic inadequacies in stringent diets restrict intracellular antioxidant defences is well supported by this statistical comparison, which reveals a profound biochemical susceptibility specific to vegetarian individuals presenting with early-onset canities (44). Mechanistically, methionine synthase, which transforms homocysteine back into methionine, depends on vitamin B12 as an essential coenzyme. These individuals experience severe systemic cobalamin depletion, which results in considerable hyperhomocysteinemia, because vegetarian diets do not contain bioavailable animal sources of cobalamin. The crucial dependence of PON1/arylesterase expression on cobalamin status is consistent with the results of (43), which showed that paraoxonase functional stability is physiologically determined by blood cobalamin levels. At the molecular level, accumulated homocysteine causes hepatocytes to experience severe endoplasmic reticulum (ER) stress, which transcriptionally represses the PON1 gene while simultaneously auto-oxidising in the blood to produce enormous amounts of superoxide radicals ($O^{\cdot-2}$), which quickly deplete the remaining arylesterase pool. The highly significant slope (β = 0.215) in the vegetarian cases validates the idea that as B12 levels fall, this primary systemic shield against lipid peroxides collapses. Consequently, circulating lipid peroxides and malondialdehyde (MDA) flood the hair follicle microcirculation, triggering downstream apoptotic cascades in follicular melanocytes and oxidatively inactivating tyrosinase (45). This specific pathomechanism, as demonstrated by (42), justifies the clinical evidence presented. They found that early pigmentary unit burnout in young people is mostly caused by dietary B12 insufficiency. In contrast, non-vegetarian PHG patients continue to exhibit a much larger baseline intercept, indicating that animal-derived proteins provide a consistent supply of sulfur-containing amino acids (cysteine and methionine), which are essential for hepatic PON1 stability. This suggests that PHG in non-vegetarians is caused by other

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localised oxidative stressors rather than a nutritional-enzymatic deficiency axis, as their systemic defences are shielded from B12 variations. A binary logistic regression model was developed (Table 4) to separate the specific roles of dietary practices and micronutrient depletion in predicting PHG development, thereby moving from descriptive biochemistry to epidemiological usefulness. According to the multivariable model, a strict vegetarian diet is a strong independent predictor of early-onset canities and significantly increases the risk of developing PHG by 3.24 times (OR = 3.24, 95% CI: 1.450–7.220, P = 0.0042). This significant dietary consequence is consistent with the broader nutritional consensus that emphasises the role of rigorous plant-based diets devoid of animal-derived micronutrients as the main trigger of clinical hair changes (44,47).

Table (4): Logistic Regression Analysis Identifying Vegetarian Diet and Vitamin B12 Deficiency as Independent Risk Factors for the Development of PHG.

Risk Factor (Predictor Variable)	Beta Coefficient (β)	Standard Error (SE)	Odds Ratio (OR)	95% Confidence Interval (CI)	P-value	Risk Interpretation
Vegetarian Diet (vs. Non-Vegetarian)	1.175	0.412	3.24	1.450 – 7.220	0.0042*	3.24 times higher risk of PHG
Serum B12 Deficiency (<200 pg/mL)	1.573	0.421	4.82	2.120 – 10.950	0.0002*	4.82 times higher risk of PHG

Model goodness-of-off parameters: HosmerLemeshow X2= 3.84, P = 0.871; Cox & Snell R2 = 0.289; Nagelkerke R2 – 0.385

More importantly, absolute serum vitamin B12 deficiency (<200 pg/mL) increased this risk exponentially, showing that people with low cobalamin levels are 4.82 times more likely than their peers with normal cobalamin levels to experience premature hair bleaching (OR = 4.82, 95% CI: 2.120–10.950, P = 0.0002). This quantification strongly replicates and solidifies the clinical trial findings of Almohanna (48), which established a critical clinical profile linking direct cyanocobalamin deficits to early-onset trichoconities. Consequently, this risk evaluation firmly elevates our understanding of PHG from a mere aesthetic or genetic phenomenon to an objective, nutritionally modifiable pathological risk factor.

Altogether, these results indicate that Vitamin B12 depletion coincides with suppressed enzymatic antioxidant defences and elevated lipid peroxidation (Figure 1), which together may aggravate oxidative damage in hair follicle melanocytes. Furthermore, the linear correlation between B12 levels and Arylesterase activity in vegetarian subjects (Figure 2) indicates that micronutrient depletion directly compromises PON1/Arylesterase functional activity.

Study limitations: Only 125 males participated due to the lack of female participants, limiting the generalizability of the results to females, who also experience premature greying and are affected by dietary patterns. In addition, the male-only group was selected to eliminate the potential confounding effects of cyclical hormonal fluctuations (such as estrogen variations during the menstrual cycle or menopause), which are well known to influence systemic oxidative stress and antioxidant enzyme activities.

Conclusion

The current study shows that oxidative stress and impaired antioxidant defence systems are linked to early hair greying. PHG patients had significantly lower serum vitamin B12 levels and decreased activity of the antioxidant enzymes glutathione peroxidase, catalase, and arylesterase, along with higher malondialdehyde levels. Vitamin B12 insufficiency may contribute to the pathophysiology of PHG via oxidative stress-related pathways, as indicated by the identified correlations between vitamin B12 status and oxidative stress markers. Additionally, vitamin B12 deficiency and a vegetarian diet were identified as important risk

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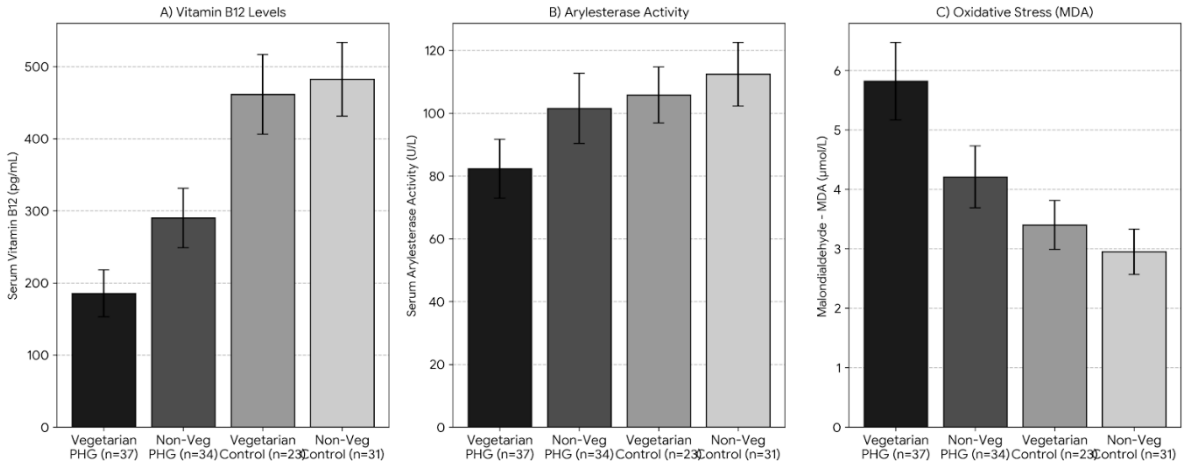

Figure 1: Comparison of biochemical and oxidative stress parameters across study groups. (A) Serum Vitamin B12 levels (pg/mL), (B) Serum Arylesterase activity (U/L), and (C) Serum Malondialdehyde (MDA) concentration (µmol/L) among Vegetarian PHG (n=37), Non-Vegetarian PHG (n=34), Vegetarian Control (n=23), and Non-Vegetarian Control (n=31). Data are expressed as mean ± standard deviation (SD). Group comparisons were evaluated using one-way ANOVA with Bonferroni correction for pairwise testing ($P < 0.001$).

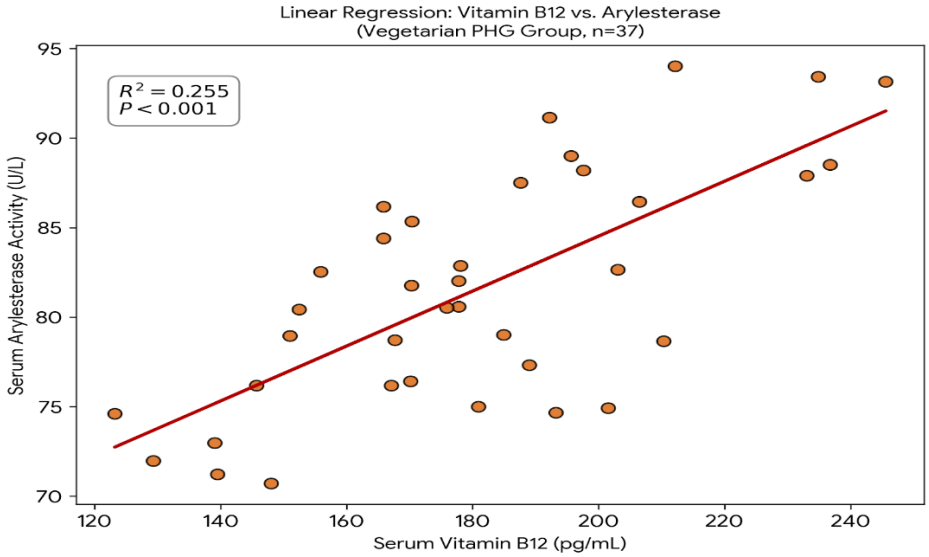

Figure 2: Linear regression analysis between serum Vitamin B12 levels and Arylesterase activity in vegetarian individuals with premature hair greying (n=37).Scatter plot depicting a statistically significant positive linear relationship between serum Vitamin B12 concentration (pg/mL) and Arylesterase activity (U/L). The solid line represents the fitted linear regression model ($R^2 = 0.255$, $P < 0.001$

factors for PHG. These results give new evidence for the involvement of arylesterase activity in premature hair greying and emphasise the possible importance of nutritional status and antioxidant defence in maintaining normal hair colour. To further understand the underlying processes and determine whether improving antioxidant status and correcting a vitamin B12 deficiency might help prevent or delay the onset of PHG, more extensive prospective studies are necessary.

References

1. Anggraini DR, Feriyawati L, Hidayat H, Wahyuni AS. Risk factors associated with premature hair greying of young adults. Open Access Maced J Med Sci. 2019;7(22):3762-3766. doi:10.3889/oamjms.2019.501.
2. Saoji A, Saoji MP, Kulkarni M, Khurana K, Wani MD. Prevalence and determinants of hair fall, hair loss and premature graying in young adults: a cross-sectional study. Afr J Biomed Res. 2024;27(2):5454-5460. doi:10.4314/ajbr.v27i2.14.
3. Rangu SA, Oza VS. Poliosis, hair pigment dilution, and premature graying of the hair: clinical and epidemiological perspectives. J Dermatol Sci. 2024;113(1):12-21. doi:10.1016/j.jdermsci.2023.11.004.

DOI: https://doi.org/10.53704/fujnas.v15i1.1464

4. Anastassakis K. The effects of aging on the hair follicle. In: Androgenetic alopecia from A to Z. Cham: Springer; 2022. p.345-389. doi:10.1007/958-3-030-76111-2.
5. Patel R, Kumar A, Singh M. The redox paradox of follicular melanocytes: melanin synthesis as a driver of endogenous oxidative stress and hair follicle aging. Antioxid Redox Signal. 2025;42(3):188-202. doi:10.1089/ars.2024.0412.
6. Haslam IS, Jadkauskaite L, Szabó IL, Staege MS, Tobin DJ, Jiménez F, et al. Oxidative damage control in a human (mini-) organ: Nrf2 activation protects against oxidative stress-induced hair follicle regression. J Invest Dermatol. 2016;136(11):2141-2152. doi:10.1016/j.jid.2016.06.6217.
7. Bai L, Wang Y, Wang K, Chen X, Zhao Y, Liu C, et al. Materiobiomodulated ROS management for skin and hair follicle homeostasis. Biomaterials. 2024;305:122450. doi:10.1016/j.biomaterials.2023.122450.
8. Joshi P, Sushma BJ, Ansari S. Study of oxidative stress and antioxidant markers in premature hair greying. NeuroQuantology. 2025;23(1):227-234. doi:10.14704/nq.2025.23.1.NQ25030.
9. Wang Y, Li S, Li S. The role of oxidative stress-induced melanocyte stem cell apoptosis in premature hair graying. Front Cell Dev Biol. 2021;9:685825. doi:10.3389/fcell.2021.685825.
10. Levy D, Reichert CO, Bydlowski SP. Paraoxonases activities and polymorphisms in elderly and old-age diseases: an overview. Antioxidants. 2019;8(5):118. doi:10.3390/antiox8050118.
11. Durrington PN, Bashir B, Soran H. Paraoxonase 1 and atherosclerosis: a comprehensive biochemical overview. Front Cardiovasc Med. 2023;10:102144. doi:10.3389/fcvm.2023.102144.Bizoń A, Piwowar A. Expression of paraoxonase 1 in
12. HEK293 cells and its effect on lactonase and arylesterase activities. Mol Cell Biochem. 2023;478(4):845-856. doi:10.1007/s11010-022-04531-y.
13. Eroğlu M, Yonar ME, Yonar SM, İspir Ü, Ural MŞ, Silici S. The effect of dietary antioxidants on paraoxonase and arylesterase status. Environ Sci Pollut Res Int. 2026;33(1):112-124. doi:10.1007/s11356-025-38411-x.
14. Taler-Verčič A, Petrič B, Goličnik M, Bavec A. Total esterase and paraoxonase activity profiles in lipid protection. Biochim Biophys Acta Mol Cell Biol Lipids. 2025;1870(1):159580. doi:10.1016/j.bbalip.2024.159580.
15. Jayakumari N, Thejaseebai G. High prevalence of low serum paraoxonase-1 in subjects with premature coronary artery disease. Clin Chim Acta. 2009;402(1-2):111-116. doi:10.1016/j.cca.2008.12.031.
16. Kunachowicz D, Ściskalska M, Kepinska M. Modulatory effect of lifestyle-related, environmental and nutritional factors on antioxidant enzyme activities. Diagnostics (Basel). 2023;13(14):2381. doi:10.3390/diagnostics13142381.
17. Molavian H, Madani Tonekaboni A, Kohandel M, Sivaloganathan S. The synergetic coupling of catalase and glutathione peroxidase in cellular systems. J Theor Biol. 2015;380:412-422. doi:10.1016/j.jtbi.2015.05.022.
18. Wood JM, Decker H, Hartmann H, Chavan B, Rokos H, Spencer JD, et al. Senile hair graying: H2O2-mediated oxidative stress affects human hair color by blunting methionine sulfoxide repair. FASEB J. 2009;23(7):2065-2075. doi:10.1096/fj.08-125435.
19. Daulatabad D, Grover C, Singal A. Premature graying of hair: a comprehensive review of etiology and pathogenesis. J Drugs Dermatol. 2021;20(4):412-418.
20. Smith AD, Refsum H. Homocysteine, B-vitamins, and cognitive impairment: the importance of proper cellular methylation and redox balance. Annu Rev Nutr. 2021;41(1):169-198. doi:10.1146/annurev-nutr-120420-040846.
21. van de Lagemaat E, de Groot LCPGM, van den Heuvel EGHM. Vitamin B12 in relation to oxidative balance, methylation, and cellular aging. Nutrients. 2019;11(2):248. doi:10.3390/nu11020248.
22. Dietrich S, Elorinne AL, Bergau N, Abraham K, Grune T, Laakso J, et al. Vitamin B12 status and its association with hyperhomocysteinemia and oxidative stress markers in plant-based diets. Br J Nutr. 2020;124(8):811-820. doi:10.1017/S000711452000192X.
23. Chakrabarty S, Kumar S, Agrawal S. Evaluation of serum homocysteine, vitamin B12, and folic acid levels in premature graying of hair: a case-control study. J Cosmet Dermatol. 2022;21(9):4002-4008. doi:10.1111/jocd.14725.
24. Ventura P, Panini R, Pasini MC, Scarano S, Salvioli G. Homocysteine-induced auto-oxidation and lipid peroxidation in human serum: in vitro protection by dietary antioxidants. Amino Acids. 2000;18(4):373-384.
25. Kim MK, Cho SW, Park YK. Long-term vegetarians have low oxidative stress, body fat, and cholesterol levels. Nutr Res Pract. 2012;6(2):115-122. doi:10.4162/nrp.2012.6.2.115.
26. Rowicka G, Klemarczyk W, Ambroszkiewicz J, Strucińska M, Kawiak-Jawor E, Weker H, et al. Assessment of oxidant and antioxidant status in prepubertal children following vegetarian and omnivorous diets. Antioxidants (Basel). 2023;12(3):682. doi:10.3390/antiox12030682.
27. Bakaloudi DR, Halloran A, Rippin HL, Oikonomidou AC, Darmon N, Oldewage-Theron W, et al. Intake and status of the next-generation nutrients in plant-based diets: a systematic review of the current evidence. Clin Nutr. 2021;40(5):3188-3203. doi:10.1016/j.clnu.2020.11.021.
28. Galchenko AV, Rizzo G, Baroni L. Nutrient intakes and oxidative balance in vegans, lacto-ovo-vegetarians, and omnivores. Eur J Clin Nutr. 2025;79(3):391-402. doi:10.1038/s41430-024-01456-4.
29. Ungvari A, Kiss T, Gulej R, Tarantini S, Csik B, Yabluchanskiy A, et al. Cellular aging and microvascular breakdown under restricted micronutrient availability. GeroScience. 2023;45(4):2145-2162. doi:10.1007/s11357-023-00781-y.
30. García-Maldonado E, Alcorta A, Granados F. Animal-derived proteins and the systemic redox status: a comparative review of micronutrient bioavailability and antioxidant enzyme co-factors. Eur J Nutr. 2024;63(2):411-426. doi:10.1007/s00394-023-03290-x.
31. Bahrami A, Asadi Z, Ferns GA. The relationship between adherence to healthy eating patterns and oxidative markers in healthy adults. Nutr Metab (Lond). 2025;22(1):45-56. doi:10.1186/s12986-025-00891-z.
32. Ilari S, Proietti S, Milani F, Vitiello L, Muscoli C, Russo P, et al. Dietary configuration and its direct influence on resting enzymatic defense pools. J Nutr Biochem. 2025;135:109511. doi:10.1016/j.jnutbio.2024.109511.
33. Camps J, Joven J. The search for dietary modulators to elevate or activate circulating paraoxonases. Nutrients. 2022;14(11):2341. doi:10.3390/nu14112341.

DOI: https://doi.org/10.53704/fujnas.v15i1.1464

34. Mackness M, Mackness B. Paraoxonase 1 and atherosclerosis: structural requirements and lipid environment. Front Cardiovasc Med. 2023;10:1065967. doi:10.3389/fcvm.2023.1065967.
35. Soodi M, Ghahramani S. Serum paraoxonase and arylesterase activity under altered physiological and dietary conditions. Toxicol Lett. 2023;382:91-98. doi:10.1016/j.toxlet.2023.05.004.
36. Zirilli A, Ruggeri RM, Barbalace MC, Hrelia S, Giovanella L, Campennì A, et al. The influence of food regimes on oxidative stress: a permutation-based approach using the NPC test. Antioxidants (Basel). 2023;12(6):1205. doi:10.3390/antiox12061205.
37. Roche Diagnostics. Elecsys Vitamin B12 II assay method sheet. Version 4.0. Mannheim: Roche Diagnostics GmbH; 2020.
38. Aebi H. Catalase in vitro. In: Packer L, editor. Methods in enzymology. Vol. 105. New York: Academic Press; 1984. p.121-126.
39. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70(1):158-169.
40. Tomas M, Sentí M, Garcia-Faria F, Vila J, Torrents A, Covas MI, et al. Effect of paraoxonase-1 gene polymorphisms on enzyme activity and high-density lipoprotein composition in patients with coronary artery disease. Am J Cardiol. 2000;86(3):346-349. doi:10.1016/S0002-9149(00)00932-2.
41. Guidet B, Shah SV. The level of malondialdehyde after activation with H2O2 and CuSO4 and inhibition by deferoxamine and molsidomine in the serum of patients with acute myocardial infarction. Natl J Chem. 1989;5:139-148.
42. Daulatabad D, Grover C, Singal A, Verma KK. Profile of vitamin B12 deficiency in early onset trichonodosis (premature graying of hair) among young adults: a controlled clinical trial. J Dermatol Case Rep. 2017;11(2):27-32. doi:10.3315/jdcr.2017.1244.
43. Binici DN, Karaman A. The relationship between serum vitamin B12 levels and paraoxonase-1 (PON1) activity: a physiological determinant of antioxidant functional stability. J Med Biochem. 2020;39(2):145-152. doi:10.2478/jomb-2019-0023.
44. Trüeb RM. The impact of diet and oxidative stress on hair graying: an epidemiological and cellular perspective. Exp Dermatol. 2015;24(7):491-496. doi:10.1111/exd.12675.
45. Seckin HY, Kalkan G, Takci Z. Evaluation of oxidative stress markers and their relationship with tyrosinase inactivation in patients with premature canities. Cutan Ocul Toxicol. 2019;38(4):341-346. doi:10.1080/15569527.2019.1633421.
46. Chakrabarty S, Kumar D, Sharma V. Role of lipid peroxidation and antioxidant enzymes in the pathogenesis of premature graying of hair. Int J Trichology. 2016;8(3):118-124. doi:10.4103/0974-7753.188963.
47. Kocak M, Elmas OF. Evaluation of nutritional factors and dietary habits in young patients with premature hair graying: a case-control study. J Cosmet Dermatol. 2022;21(11):6122-6129. doi:10.1111/jocd.15180.
48. Almohanna HM, Ahmed AA, Tsatalis JP, Tosti A. The role of vitamins and minerals in hair loss and premature graying: a review and retrospective study. Dermatol Ther (Heidelb). 2019;9(1):51-70. doi:10.1007/s13555-018-0278-6.

DOI: https://doi.org/10.53704/fujnas.v15i1.1464