

Research Article

Evaluation of the Role of Galanin Hormone and Biochemical Markers in Individuals with Hair Loss

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Abstract: Hair loss is one of the problems that many people suffer from without knowing the real reason. Galanin is a neuropeptide closely involved in the modulation and inhibition of action potentials in neurons in the brain, spinal cord, and gut of humans. Therefore, we believe that it has a role in mineral absorption and hair loss. The aim of this study is to evaluate the level of galanin and its relationship with the levels of some minerals in the serum of people suffering from hair loss compared to healthy people. There are 60 patients who have lost their hair and 30 healthy people. This study discovered that the mean serum Galanin and Troponin level were higher in patients with hair loss compared to the control group. The findings were significant ($p < 0.001$). As well as the Prolactin and FSH levels were higher in patients with hair loss compared to the control group. The findings were significant ($p < 0.001$). While the TSH, Zn, Mg, Cu, Fe and Ca levels were lower in patient with hair loss compared with the control group, the result was significant ($p < 0.001$). Also, the LH levels were increased in patient with hair loss compared with the control group, but the result was non-significant ($p > 0.05$). The results of this study that increasing the level of Galanin and Troponin has an effect on hair loss, and also that the hormone prolactin affects the growth of hair follicles, as well as the hormone LH, which works to increase the level of testosterone, and this contributes to hair loss.

Keywords: Galanin, Zn, Fe, Mg, Cu, Hair Loss

Introduction

One of the most frequent symptoms from female patients referred to dermatology clinics is Female Pattern Hair Loss (FPHL). It is the most frequent cause of alopecia, affecting 6–12% of women in their 20s and 30s and more than 55% of those over 70. Clinically, FPHL is defined by widespread, non-scarring hair loss in the frontal, central, and partial lobes of the scalp without visible hair thinning. The frontal hairlines are preserved in their typical way. Although the etiology of FPHL is still unclear, it most likely results from a complex hereditary characteristic. Mechanisms not related to androgens might be involved in this trait (Dhaher and Alwan, 2020). The frontal and vertex parts of the body have the most hair density loss in FPHL, although the parietal and occipital

regions may also be affected. FPHL is a diffuse, progressive, non-scarring alopecia. The pathogenesis of FPHL is the miniaturization of hair follicles brought on by a gradual reduction in the length of each hair cycle. Terminal hair follicles undergo a transformation into vellus-like follicles, which have a shorter hair cycle due to a reduction in their anagen phase, resulting in small, thin hair shafts. The prevalence of FPHL in women varies by age, with 12% affected between 20 and 29 years old, 41% by 69 years old, and over 50% by 79 years old (Malakar *et al.*, 2019). There are two sections to the structure of a hair follicle: the upper and bottom sections. The bulb and suprabulbar area are the terms used to describe the lower portion, while the infundibulum and isthmus comprise the upper part. The hair matrix, which is made up of quickly reproducing keratinocytes, and the

dermal papilla, which consists of a collection of specialized fibroblasts, blood capillaries, and nerve terminals, construct the hair bulb. The subcutis serves as an anchor for the scalp hair follicles, which go through repeated growth cycles (Schneider *et al.*, 2009).

Galanin is a neurotrophic factor that is present in both the central and peripheral nervous systems and is widely distributed in human skin. Nevertheless, the precise distribution and function of galanin in the Hair Follicle (HF) are yet to be fully understood. They contribute to metabolism, either directly or indirectly, and have crucial functions in regulating it. Metals are essential for the initiation and functioning of approximately 25% of the enzymes in the body. Galanin (GAL) is a neuropeptide that is extensively found in both the central and peripheral nervous systems. The human hair follicle has been recognized as both an origin and a recipient of GAL. This neurotransmitter is known to impede human hair development by reducing the proliferation of matrix keratinocytes, shortening the anagen phase, and decreasing hair shaft elongation (Grymowicz *et al.*, 2020).

Calcium, copper, iron, magnesium, and zinc are essential elements for optimal health. Research has demonstrated that calcium facilitates the body's process of breaking down fat and metabolizing carbohydrates. Copper is a necessary cofactor for several enzymes, such as cytochrome oxidase, which is responsible for the oxidation of fatty acids in mitochondria (Banach *et al.*, 2020). observed a positive correlation between serum copper levels and BMI in overweight persons. Iron binds with hemoglobin and myoglobin to facilitate the transportation of oxygen throughout the body. Iron is a vital constituent of cytochrome c, which plays a crucial role in transmitting electrons inside the mitochondria. Iron deficiency is common in overweight and obese persons. Magnesium has a role in controlling the metabolism of lipids and glucose (Silva *et al.*, 2019). Hair has shown to be a valuable tool in forensic medicine, as well as in screening for heavy metal intoxication and monitoring pollution. Nevertheless, there have been doubts over its effectiveness in identifying certain illnesses or assessing nutritional condition (Banach *et al.*, 2020). Insufficient evidence exists on the association between mineral and trace element levels, Body Mass Index (BMI), and age distribution in adult females when analyzing their hair. Our previous investigation into hair and blood determined that they both serve as indicators of the body's levels of arsenic, copper, iron, selenium, and zinc. Conventional hematological tests have the capability to detect the amounts of calcium (Ca), vitamin D3, iron (Fe), magnesium (Mg), and Zinc (Zn) in the blood either on the day of testing or within the preceding 2-3 days. Thus, instead of doing a hematological examination, the research advocated using hair analysis to examine the distribution ranges of Ca, D3, Fe, Mg, K, and Zn levels in

the hair of adult females and their correlation with BMI (Funes *et al.*, 2023). Female pattern hair loss is a condition where the hair gradually becomes thinner due to the shrinking of hair follicles, without any scarring. There is evidence of a 12% occurrence rate in females around 30 years old and a 40% occurrence rate in females approximately 60 years old (Larvie *et al.*, 2019). The precise mechanism responsible for the follicular shift observed in female pattern hair loss remains incompletely understood. The heightened responsiveness of the hair follicle to typical androgen levels may elucidate the mechanism by which the disorder arises in individuals lacking hyperandrogenism. Alopecia areata has been linked to several illnesses, including as type 1 diabetes, hyperlipidemia, and atherosclerosis. These disorders all contribute to an elevated risk of cardiovascular disease (Aderibigbe *et al.*, 2011). Prior research has established a correlation between female-pattern hair loss and the risk of cardiovascular disease (Grymowicz *et al.*, 2020). Female-pattern hair loss is linked to dyslipidemia, obesity, insulin resistance, hypertension, and metabolic syndrome (Ishak and Piliang, 2013). Heart troponins are involved in the contractile mechanism of cardiac myocytes. Following a myocardial infarction, there is a significant rise in the plasma concentration of cardiac troponin I as a result of the death of cardiac cells. This increase in troponin I levels serves as a dependable indicator of damage to cardiac myocytes (Vujovic and Del Marmol, 2014; Lim *et al.*, 2018). Elevated levels of cardiac troponin I were seen in individuals with autoimmune diseases, even in the absence of heart failure, suggesting the presence of subclinical myocardial damage (Arias-Santiago *et al.*, 2010). This evaluation offers a thorough and current comprehension of the impact of hormones on hair follicles. This study underscores the substantial advancements made in the investigation of the impact of hormone fluctuations on hair at different phases of women's lives in recent years (Herskovitz and Tosti, 2013; Fabbrocini *et al.*, 2018).

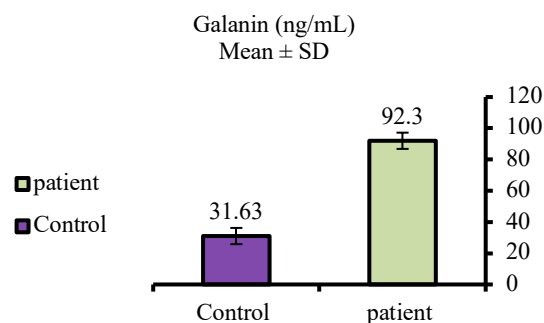


Fig. 1: Comparison between patient with hair loss and Control group regarding the mean \pm SD of galanin ($p < 0.009$)

Materials and Methods

Study Sample

This case-control hospital-based study was done in Tikrit City/Iraq for patients who underwent a Coronary Care Unit at Tikrit Teaching Hospital from the period between 1st January to 15th June 2024. This study included 60 hair loss sufferers and 30 healthy adults with no underlying diseases or alopecia as a control group Based on sample size calculation by G-Power analysis (see Figs. 3-4). Individuals who had used copper or zinc supplements within the last 6 months, no prior hair loss family history and no had underlying health conditions (e.g., hypothyroidism, PCOS, iron-deficiency anemia) as well as those who had sought treatment at other hospitals specifically for hair loss, were excluded from both the patient and control groups.

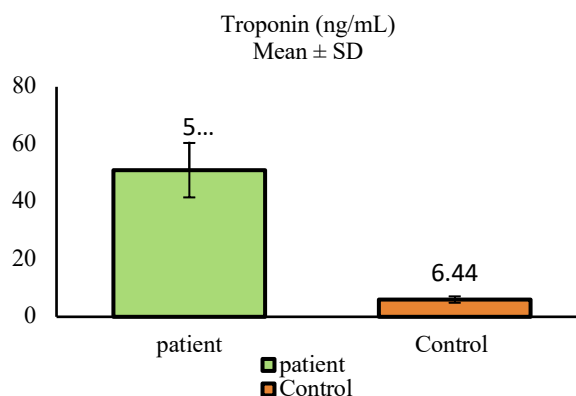


Fig. 2: Comparison between patient with hair loss and Control group regarding the mean \pm SD of Troponin ($p < 0.0002$)

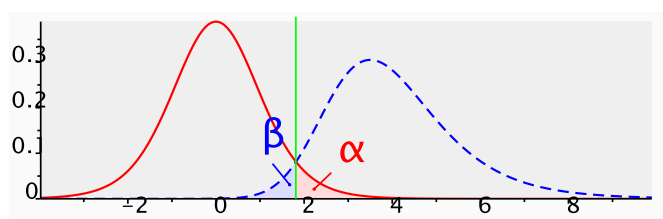


Fig. 3: G power analysis (29)

t test – Means: Difference between independent means (two groups)
 Tail(s) = One, Allocation ratio $N2/N1 = 2/1$, α err prob = 0.05, Effect size $d = 2.2802$

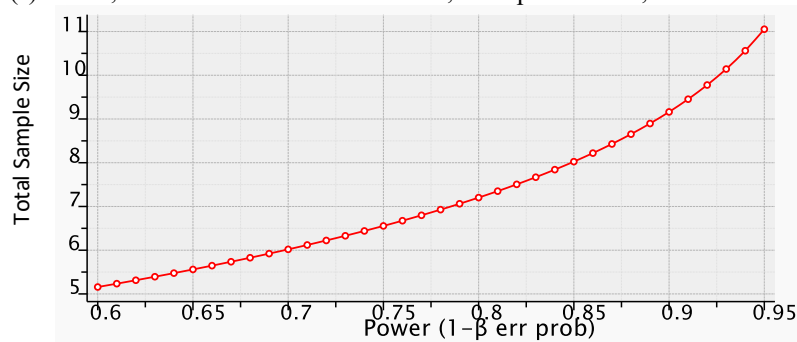


Fig. 4: G power analysis X-Y plot for a range of Values

Data and Sample Collection

All participants completed an extensive medical checkup and submitted questionnaires regarding their health and lifestyle upon enrolment. Blood samples were obtained from subjects between 7:00 AM and 2:00 PM. Approximately five millilitres of blood were obtained from the antecubital veins of both patients and controls into gel tubes devoid of anticoagulants, maintained at room temperature for 10-15 minutes to facilitate clotting, and subsequently centrifuged at 3000 rpm for 15 minutes. The transparent serum was transferred into clear, dry

Eppendorf tubes for each sample and preserved at -80°C until analysis for Zn, Fe, Mg, Cu, VitD3, Ca, TSH, prolactin, LH, FSH, Troponin, and galanin. Samples from the control subjects were collected and processed in the same way. The biomarkers Vit D3, TSH, prolactin, LH, FSH, Troponin, and galanin were quantified using the enzyme-linked immunosorbent assay (ELISA) with a commercially available human kit (Bioassay Technology Kit), while the biomarkers Zn, Ca, Fe, Mg, and Cu were assessed via inductively coupled plasma mass spectrometry (ICP-MS, Elan DRC-e; PerkinElmer Inc., Waltham, MA, USA).

Statistical Analysis

The statistical analysis was carried out with the help of SPSS, which stands for Statistical Package for the Social Sciences, version 25 (SPSS, Chicago). When dealing with continuous data, the Shapiro-Wilk test was utilised.

Results and Discussion

Distribution of Hair Loss According to Age

The total number of subjects that participate are 60 patients with hair loss. This study showed that the peak age with hair loss was between 20–30 years and its percentage was 50%, while the least age group 41-50 years and its percentage which was found to be 20%.

Distribution of Hair Loss According to Sex

The total number of subjects that participate are 60 patients with hair loss. This study showed that Female with hair loss its percentage was 67%, while Male its percentage which was found to be 33%.

Hair loss, which affects both males and females, can cause significant psychological distress. The lack of necessary micronutrients in one's diet has been linked to the condition of the hair and the occurrence of hair loss. There is little doubt that diet plays a role in hair loss conditions (Weil *et al.*, 2017), and taking food supplements that target several components involved in this complicated cause can aid in managing hair loss.

Galanin Level in Patient with Hair Loss

As shown in Table 1, the mean of the serum level of galanin in patient with hair loss comparing with the control group (92.3±29.2 versus 31.63±12.84). The result was significant (p<0.009).

Galanin is a neuropeptide present in the central and peripheral nervous systems. GAL has been identified as both a source and a target within human hair follicles. The recently discovered neurotransmitter acts as an inhibitor of human hair growth by restricting the proliferation of matrix keratinocytes, shortening the anagen phase, and decreasing the length of the hair shaft (Holub *et al.*, 2012). Hormones such as galanin, crucial for terminal hair growth, significantly influence the hair cycle and the architecture of hair follicles. They convert fine, straight, light vellus hairs into robust, darker terminal hairs in regions of the body that are characteristic of each sex. They bind to the intracellular androgen receptors of the dermal papilla cells in hair follicles. The intracellular enzyme 5-alpha reductase is essential for the majority of hair follicles to convert testosterone into DHT (Grymowicz *et al.*, 2020).

Troponin Level in Patient with Hair Loss

As shown in Table 2, the mean of the Troponin levels were increased in patient with hair loss comparing with the control group (51.4±18.56 versus 6.44±1.20). The result was significant (p<0.0002).

Each of these coexisting medical conditions augments the probability of developing cardiovascular disease (Kabir and Goh, 2013).

Table 1: Comparison between patient with hair loss and Control group regarding the mean ± SD of Galanin

Study groups	n	Galanin (ng/mL) Mean ± SD	P. value
patient with hair loss	60	92.3±29.2	<0.009 [S]
Control group	30	31.63±12.84	

T-test was *: significant at p≤0.05

SD: Standard Deviation; S: Significant; NS = Non-Significant

Table 2: Comparison between patient with hair loss and Control group regarding the mean ± SD of Troponin

Study groups	N	Troponin (ng/L) Mean ± SD	P. value
patient with hair loss	60	51.4±18.56	<0.0002 [S]
Control group	30	6.44±1.20	

T-test was *: significant at p≤0.05

SD: Standard Deviation; S: Significant; NS = Non-Significant

Female pattern hair loss is distinguished by a reduction in hair density throughout the entire head and frontal scalp, but the hairline at the front remains unaffected. It is the predominant form of alopecia, impacting up to 50% of women throughout the course of their lifetimes. Cardiac troponin I is present in myocardial tissue. heart troponin levels can indicate several pathological occurrences, such as the death of heart muscle cells and programmed cell death. Evaluating the levels of troponin in the

bloodstream is suitable for predicting both immediate and future negative consequences (Shoeib *et al.*, 2021).

TSH, Prolactin, LH and FSH Levels in Patient with Hair Loss

As shown in Table 3, the mean of the serum TSH level were decreased in patient with hair loss comparing with the control group (0.0572±0.021 versus 1.872±0.01) respectively. The result was significant (p<0.01). While the prolactin levels were increased in patient with hair loss

comparing with the control group (23.9±4.4 versus 19.9±2.4) respectively at (p<0.01). as well as FSH in patient with hair loss as compared with the control group (25.1±1.2 versus 13.1±1.2) respectively at (p<0.01). Also, the LH levels were increased in patient with hair loss comparing with the control group (14.1±2.2 versus 13.1±2.2) respectively, but the result was non-significant (p>0.06).

Thyroid hormones play an important role in hair growth, which means that hair loss could be a sign of thyroid dysfunction. Subtle changes in TSH levels in the blood significantly impact the growth of hair follicles on the scalp. Low TSH levels can cause slow-growing, coarse, dry, brittle hair. This imbalance may be associated with hair loss, which suggests that low TSH levels in patients are one of the causes of hair loss (Mazhar *et al.*, 2021). Prolactin were higher in patients compared to healthy controls, which led to a significant inhibition of hair shaft elongation and early catagen development, along with a decrease in proliferation of keratinocytes in the hair follicle and an increase in their programmed death (Grymowicz *et al.*, 2020). Correspondingly, serum LH and serum FSH levels were markedly elevated in contrast to the control group. Cohen *et al.* also observed elevated LH levels, consistent with our results (Pathak *et al.*, 2023). Narad *et al.* did not demonstrate a significant difference in the levels of LH and FSH among patients with androgenetic alopecia (Narad *et al.*, 2013). Elevated LH results in heightened testosterone levels, hence contributing to the exacerbation of male pattern baldness.

Furthermore, LH induces the adrenal gland to synthesise androstenedione, a less potent androgen. Elevated LH levels indicate that the hypophyseal-adrenal axis plays a role in the pathophysiology of premature androgenetic alopecia.

D3 and Ca Levels in Patient with Hair Loss

Table 4 illustrates that the mean serum level of D3 in patients with hair loss is significantly lower than that of the control group (7.8911±0.61 versus 44.872±0.01, respectively). The outcome was statistically significant (p<0.001). Calcium levels in patients with hair loss were compared to the control group, yielding values of 5.1389±0.41 versus 9.9±1.4, respectively. The outcome was statistically significant (p<0.01). Endocrine disorders, including hypercortisolism and excessive growth hormone secretion, can lead to various hair-related issues, such as different forms of alopecia, female pattern hair loss, and hirsutism. Hirsutism is a prevalent hormonal disorder that impacts 5-10% of women during their reproductive years. It is characterised by the prevalence of terminal hair in a pattern commonly observed in males, manifesting in females.

Multiple investigations have shown a clear correlation between calcium and D3 levels in the body and the metabolism of fatty acids in brown adipose tissue located in subcutaneous layers (Tombuloglu *et al.*, 2020; Trieu and Eslick, 2014).

Table 3: Serum Level of TSH, prolactin, LH and FSH in patient with hair loss and Control group

Biomarker	Mean ± SD		p. Value
	Patient group N = (60)	Control group N = (30)	
TSH (mIU/L)	0.0572±0.021	1.872±0.01	<0.01 [S]
Prolactin (ng/mL)	23.9±4.4	19.9±2.4	<0.01[S]
LH (IU/L)	14.1±2.2	13.1±2.2	>0.06 [NS]
FSH (mIU/mL)	25.1±1.2	13.1±1.2	<0.01[S]

T-test was *: significant at p<0.05

SD: Standard Deviation; S: Significant; NS = Non-Significant

Table 4: Serum Level of D3 and Ca in patient with hair loss and Control group

Biomarker	Mean ± SD		p. Value
	Patient group N = (60)	Control group N = (30)	
Vit. D3 (ng/mL)	7.8911±0.61	44.872±0.01	<0.001 [S]
Ca (mg/dL)	5.1389±0.41	9.9±1.4	<0.01 [S]

T-test was *: significant at p<0.05

SD: Standard Deviation; S: Significant; NS = Non-Significant

Elevated levels of calcium in the body impede the generation of fresh adipose tissue and promotes the degradation of pre-existing fat. While the primary impact of the sickness is on the skin, there are several associated medical conditions. Alopecia areata has been correlated

with several illnesses, such as type 1 diabetes, hyperlipidemia, and atherosclerosis. Several endocrine disorders, such as thyroid dysfunction, hyperandrogenism, and hyperprolactinemia, can affect women's reproductive cycles.

Zn, Fe, Mg and Cu Levels in Patient with Hair Loss

As shown in Table 5, the mean of the serum Zn levels were decreased in patient with hair loss comparing with the control group (36.07±4.62 versus 78.12±13.56) respectively. The result was significant (p<0.001). Also, the Fe levels were decreased in patient with hair loss comparing with the control group (30.6±5.4 versus 93.3±28.9) respectively. The result was significant (p<0.001). As well as Mg levels were decreased in patient with hair loss comparing with the control group (0.64±0.02 versus 1.8±0.12) respectively. The result was significant (p<0.001). Additional Cu levels were decreased in patients with hair loss comparing with the control group (47.4±9.2 versus 89.1±18.2) respectively. The result was significant (p<0.01).

Zinc, serving as a cofactor for metalloenzymes, is essential for nearly all metabolic activities in the body, including hair development. Moreover, it is crucial to the hair follicle cycle. Recent studies indicate that iron (Fe), magnesium (Mg), and zinc (Zn) significantly influence the differentiation and proliferation of dermal papilla cells. These cells are specialised fibroblasts that are essential for the development of hair follicles (Willeit *et al.*, 2017). The potential involvement of zinc, iron, and magnesium in the development of hair loss has been suggested. However, inconsistent findings have been reported in clinical studies about their actual association. During a recent experiment, individuals with blood zinc

levels equal to or less than 70 µg/dl were administered a daily dose of 50 mg of zinc gluconate for a duration of 12 weeks (Pincemail and Meziane, 2022). The administration of zinc resulted in a mean rise in serum zinc levels of 27.6 µg/dl, with a notable improvement observed in 60% of patients. Comparable research found no disparities in zinc, iron, or magnesium, except for significantly elevated magnesium levels of serum. While there may be a strong theoretical connection between blood copper levels and hair loss, analogous to zinc, several investigations have produced contradictory findings (Trüeb, 2020).

In an Indonesian investigation, the blood levels of zinc and magnesium were relatively stable among 60 patients and 30 individuals in a normal control group. However, there was a substantial decrease seen in the zinc levels. Nevertheless, a study unveiled that the patient group had reduced serum copper levels (Fiorentini *et al.*, 2021). According to this research, it is advised to assess the levels of Fe, Mg, and zinc instead of copper. If they find their levels are too low, supplements are recommended to compensate for the deficiency. While the present study did not aim to assess the levels of zinc and copper in hair, it is crucial to do extensive investigations on zinc concentrations in both serum and hair to determine the need for zinc supplementation in the future (Limketkai *et al.*, 2022).

Table 5: Serum Level of Zn, Fe, Mg and Cu in patient with hair loss and Control group

Biomarker	Mean ± SD		p. Value
	Patient group N = (60)	Control group N = (30)	
Zn (µg/dL)	36.07±4.62	78.12±13.56	<0.001 [S]
Fe (µmol/L)	30.6±5.4	93.3±28.9	<0.001[S]
Mg (mg/dL)	0.64±0.02	1.8±0.12	<0.001[S]
Cu (mg/dL)	47.4±9.2	89.1±18.2	<0.01[S]

T-test was *: Significant at p≤0.05

SD: Standard Deviation; S: Significant; NS = Non-Significant

Conclusion

The skin can be regarded as an endocrine gland due to its ability to produce a wide range of hormones and express corresponding hormone receptors.

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Authors Contributions

Sheerin Farouq Shaker: Conceptualization, methodology, validation, investigation, writing original draft.

Sarab Dalaf Khalaf: Conceptualization, formal analysis, resources, writing original draft.

Omeed Akbar Ali: Conceptualization, methodology, validation, investigation, resources, writing review and edited.

Shaimaa Tariq Mahmood: Methodology, resources, writing review and edited.

Ethics

The Ethical Committee of the College of Medicine at Tikrit University issued approval for this study on December 24, 2023, with the number 68/240. This study was carried out in accordance with ethical standards. Before joining in the study and taking blood samples from patients, the participants and their guardians gave their agreement to take part in the medical research.

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Correlations

		Galanin	Prolactin	Ca	Zn	Fe	Mg	LH	Cu
Galanin	Pearson Correlation	1	-.685**	.008	.253	.006	-.151-	-.524**	-.068-
	Sig. (2-tailed)		.000	.954	.068	.967	.280	.000	.629
	N	60	60	60	60	60	60	60	60
Prolactin	Pearson Correlation		1	.093	-.162-	-.221-	.115	.786**	.211
	Sig. (2-tailed)			.509	.246	.111	.410	.000	.129
	N		60	60	60	60	60	60	60
Ca	Pearson Correlation			1	-.081-	-.024-	-.115-	.001	-.259-
	Sig. (2-tailed)				.566	.863	.414	.992	.062
	N			60	60	60	60	60	60
Zn	Pearson Correlation				1	.321*	-.189-	-.081-	-.003-
	Sig. (2-tailed)					.019	.175	.563	.981
	N				60	60	60	60	60
Fe	Pearson Correlation					1	.151	-.254-	.025
	Sig. (2-tailed)						.279	.067	.859
	N					60	60	60	60
Mg	Pearson Correlation						1	.191	.270
	Sig. (2-tailed)							.172	.050
	N						60	60	60
LH	Pearson Correlation							1	.213
	Sig. (2-tailed)								.126
	N							60	60
Cu	Pearson Correlation								1
	Sig. (2-tailed)								
	N								60

** . Correlation is significant at the 0.01 level (2-tailed)

* . Correlation is significant at the 0.05 level (2-tailed)