ANALYSIS OF ELEMENTAL COMPOSITION FROM NON-DIABETIC AND DIABETIC PATIENTS HAIRS, NAILS AND BLOOD USING SCANNING ELECTRON MICROSCOPY (SEM) AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDS)

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ABSTRACT: There is mounting evidence that diabetes mellitus alters, how trace elements are broken down, and that these nutrients may be especially important in the onset and course of the condition. Comparing the locations of essential and trace elements was the aim of the current investigation. The typical levels of substances found in the human body include Calcium (Ca), Potassium (K), Phosphorus (P), Magnesium (Mg), Manganese (Mn), Sodium (Na), Oxygen (O), and Zinc (Zn). In biological samples (blood, nails, and scalp hair), individuals with type 1 diabetes mellitus (n = 6) were compared to non-diabetic control subjects (n = 6), aged between 35 and 60 of both genders. The concentration of the elements was assessed through Energy Dispersive Spectroscopy (EDS) in a Scanning Electron Microscope (S.E.M). The accuracy and precision were confirmed by the Critical point drying technique and by utilizing verified reference materials. The total recoveries of all components were observed to be (97.60-99.49) of verified values. The findings of this research indicated that the average levels of Ca, K, P, Mg, Mn, Na, O, and Zn were notably lower in blood, nails, and scalp hair samples of diabetic individuals when compared to control subjects of both sexes. These findings align with those obtained in other research indicating that lack and effectiveness of certain vital trace metals may contribute to the onset of diabetes mellitus. For the control of Diabetic Mellitus, I suggested different plants like *Chicorium Intybus L., Eugenia Jambolina Lam/ Syzigium Cumini L., Citrus Decumana L.* and *Withania Coagulans Dunal.*

Keywords: Medicinal Plants, Essential Elements, Hypoglycemic agents, Diabetes mellitus and Age groups.

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INTRODUCTION

Ancient Greece and Rome are where the term "diabetes mellitus" first appeared. The Latin word "mellitus" means sweet, while the Greek word "diabetes" describes the flow of fluids. After ancient societies discovered a link between the illness and the sweet taste of urine, this nomenclature was created. Millions of people around the world suffer from diabetes, a common chronic illness. It ranks as the seventh most common cause of death in the United States. Chronic diabetes complications can result in microvascular and macrovascular problems, as well as harm to organs and tissues. Retinopathy, nephropathy, neuropathy, ischemic heart disease, and cerebrovascular disease are some of these side effects [1]. Worldwide, diabetes affects people of all ages, according to the World Health Organization. About 180 million people worldwide suffer from type 2 diabetes, formerly known as adult-onset diabetes, which makes up over 95% of all cases of the disease. By 2030, this figure is anticipated to increase. Furthermore, about 6% of deaths worldwide in 2000 were attributable to complications from diabetes. [2]. The International Diabetes Federation estimates that 33 million cases of diabetes will be reported in Pakistan in 2022, making up 26.7 percent of the country's adult population. [3]A metabolic disease called diabetes mellitus is typified by high blood sugar levels brought on by problems with the effectiveness or production of insulin. A number of organs, including the kidneys, heart, blood vessels, nerves, and eyes, can suffer harm, malfunction, and failure as a result of persistently elevated blood sugar levels. [4]. While long-term metabolic abnormalities frequently cause irreversible alterations to the body's cells, especially affecting the vascular system, severe metabolic disturbances can be lethal. Unusual elevated blood glucose levels are a defining feature of diabetes. Because it affects enzyme function and forms zinc-enzyme complexes, zinc is essential for metabolism. [5] The most prevalent trace element in the human body, zinc is essential for many physiological functions. It influences multiple metabolic pathways by acting as a cofactor for a large number of transcription factors and enzymes. Numerous chronic illnesses, such as diabetes, metabolic syndrome, and related comorbidities, have been connected to zinc imbalance. [6] Growth impairment, immunological disorders, and taste dysfunction can result from a zinc deficiency. Furthermore, it has been demonstrated that a zinc deficiency raises the risk of diabetes. [7] The human body's fourth most abundant element, magnesium, is necessary for basic biological processes like energy metabolism and DNA synthesis. It is also essential for the phosphorylation and glucose metabolism processes. Insulin resistance, glucose intolerance, dyslipidemia, and complications from diabetes have all been linked to magnesium deficiency [8]. Lack of magnesium may be a factor in endothelial damage and insulin dysfunction. For diabetic patients, hypomagnesemia should also be taken into account when assessing neuromuscular dysfunction because it can exacerbate muscle weakness. [9] It is unclear how trace element deficiencies relate to the onset of disease. On the other hand, rigorous metabolic control is frequently thought to postpone the development of late complications. Comparing the levels of different components in diabetic patients and healthy individuals was the goal of this study [10]. Determining the state of these components in diabetic patients is crucial to comprehending their function in managing the condition.

METHOD AND MATERIAL

Selection criteria for patients: This study included individuals aged 35 to 60 years of both genders, who were then divided into two groups. The controls comprised Group-1, while those with diabetes for over 10 years were classified as Group 2, all of whom are not consuming any trace element supplements. Samples with hemolysis, jaundice, cardiovascular disease (CVD), and renal failure were not included.

The Ucheck glucometer was used to measure the glucose level. This approach was utilized due to its precision, dependability, and straightforwardness.

SEM Sample Preparation Procedure: The preparation of SEM samples primarily relies on the characteristics of the sample, and it may require some or all of these steps.

➢ Washing and cutting > Fixation and stabilization > Dehydration > Dehydrating > Mounting on a stub > Coating with a conductive material

SEM/EDS Process: The materials utilized include regular paper tape, double-sided carbon tape, and a metallic stub, while for the analysis, we used Hair, Nails, and Blood from both a healthy individual and a diabetic mellitus patient. The time projected for blood analysis ranges from 12:20 to 4:00. In a scanning electron microscope (S.E.M), electrons are generated by creating a vacuum after 20 minutes. Subsequently, we examined hair and nails, then soaked the samples in methanol and chloroform (2:1). Following this step, we rinsed the samples in distilled water three times to eliminate impurities, and finally placed the samples in a cylindrical incubator at 80℃ for sterilization. The current research involved the measurement of fasting blood sugar and elemental levels in blood samples from 6 individuals of both genders, including 6 healthy individuals without diabetes as controls and 6 diabetes mellitus patients as cases.

Elemental Examination of Hairs, Nails and Blood Samples via SEM (Scanning Electron Microscope). Analysis of Elements was done in JEOL JSM-6490LV scanning electron microscope (S.E.M) by using a BRUKER E.D.S model at 1000℃-degree vacuum in Energy Dispersive Spectroscopy (EDS). We use 200 quantex software for chemical analysis. And we use chiller from 18℃ to 20℃ for getting better result.

SEM/EDS In scanning electron microscope at solid specimen's surface various types of signals generated by the focused beam, which possesses electron highly energized. These signals contains or unveil the sample's information. It Includes:

(1) Morphology: About the size and as well as about the shape of the particles that compose the sample.

(2) Composition: About the composition of compounds and elements and also their approximate amount.

(3) Surface Topography: The features relate to their surface, how it look and its texture.

(4) Crystallographic Information: About the arrangement of atoms in the sample. With the help of EDS (Energy Dispersive X-Ray Spectrometer) various types of elements determination takes place like environmental samples and different types of trace elements etc. Various elements morphological characters also determined through this technique.

RESULTS

The elemental analysis of blood, hair, and nail samples shown perfect changes between diabetic patients and non-diabetic controls (Tables 1–3, Figs. 1–3).

Calcium levels in blood samples from diabetics were significantly lower (mean 7.63 ± 1.69 mg/dL) than those from controls (8.6–9.6 mg/dL). Additionally, diabetics had consistently lower magnesium levels (0.4–1.6 mg/dL) than non-diabetics (1.7–2.1 mg/dL). Although there were fluctuations in phosphorus levels, diabetics generally had slightly lower averages (3.98 ± 1.76 mg/dL). It's interesting to note that potassium exhibited the opposite pattern, with much higher concentrations in diabetics (7.1–9.9 mEq/L) than in non-diabetics (3.1–4.4 mEq/L). Oxygen and manganese levels varied but generally decreased in diabetic blood. Interestingly, zinc was present in healthy individuals (87–111 µg/dL) but completely absent in all diabetic blood samples, suggesting a severe elemental deficiency. Similar trace element depletion patterns were seen in hair samples; in diabetics, calcium was reduced to 25–178 µg/g compared to 210–565 µg/g in non-diabetics, and potassium and phosphorus were reduced to 11.83 ± 5.69 µg/g and 122.33 ± 39.64 µg/g in diabetics, respectively, versus 15–19 µg/g and 145–179 µg/g in controls. Magnesium, sodium, and manganese concentrations were consistently lower in diabetic hair. Zinc was present in all samples, but was significantly lower in diabetics (33–97 µg/g) versus controls (156–175 µg/g). The elemental imbalance was most pronounced in the nail samples, where calcium concentrations in diabetics ranged only from 250 to 352 µg/g while controls showed extremely high values (1900 to 2600 µg/g). Potassium was also severely depleted in diabetics (150 to 199 µg/g) compared to controls (1500 to 1800 µg/g). Phosphorus and magnesium followed the same trend, averaging 282.92 ± 247.16 µg/g and 88.33 ± 80.69 µg/g in diabetics, far below control values (456 to 754 µg/g and 98 to 252 µg/g, respectively). Manganese was nearly absent in diabetics (0.04 to 0.20 µg/g) compared with normal values of 3.0 to 4.9 µg/g. Sodium and zinc were also dramatically reduced in diabetics (Na: 135 to 199 µg/g; Zn: 32 to 95 µg/g) compared with controls (Na: 1100–1356 µg/g; Zn: 109–240 µg/g).

Table: 1 Elemental Values in Blood

Group	Age	Ca (mg/dL)	K (mEq/L)	P (mg/dL)	Mg (mg/dL)	Mn (µg/L)	O (mmHg)	Zn (mcg/dL)
D. Patient 1	46	7.1	8.9	1.0	0.9	19	92	-
Non-Diabetic 1	45	8.8	3.9	3.3	1.8	9	96	90
D. Patient 2	51	5.8	9.9	5.7	0.4	5	89	-
Non-Diabetic 2	51	8.9	4.1	3.2	1.9	7	98	110
D. Patient 3	35	7.1	9.6	4.6	1.6	23	97	-
Non-Diabetic 3	36	9.5	4.4	3.3	1.9	5	97	109
D. Patient 4	37	4.8	7.1	7.7	1.1	19	96	-
Non-Diabetic 4	35	9.2	4.1	3.9	1.7	6	97	99
D. Patient 5	56	6.6	8.8	2.1	1.2	3	95	-
Non-Diabetic 5	57	8.6	3.1	4.1	2.1	6	96	111
D. Patient 6	49	5.5	7.9	5.5	0.9	19	93	-
Non-Diabetic 6	50	9.6	3.1	3.3	1.9	6	97	87
Mean ± SD		7.63 ± 1.69	6.24 ± 2.69	3.98 ± 1.76	1.45 ± 0.53	10.58±7.17	95.27±2.72	101.0±9.58

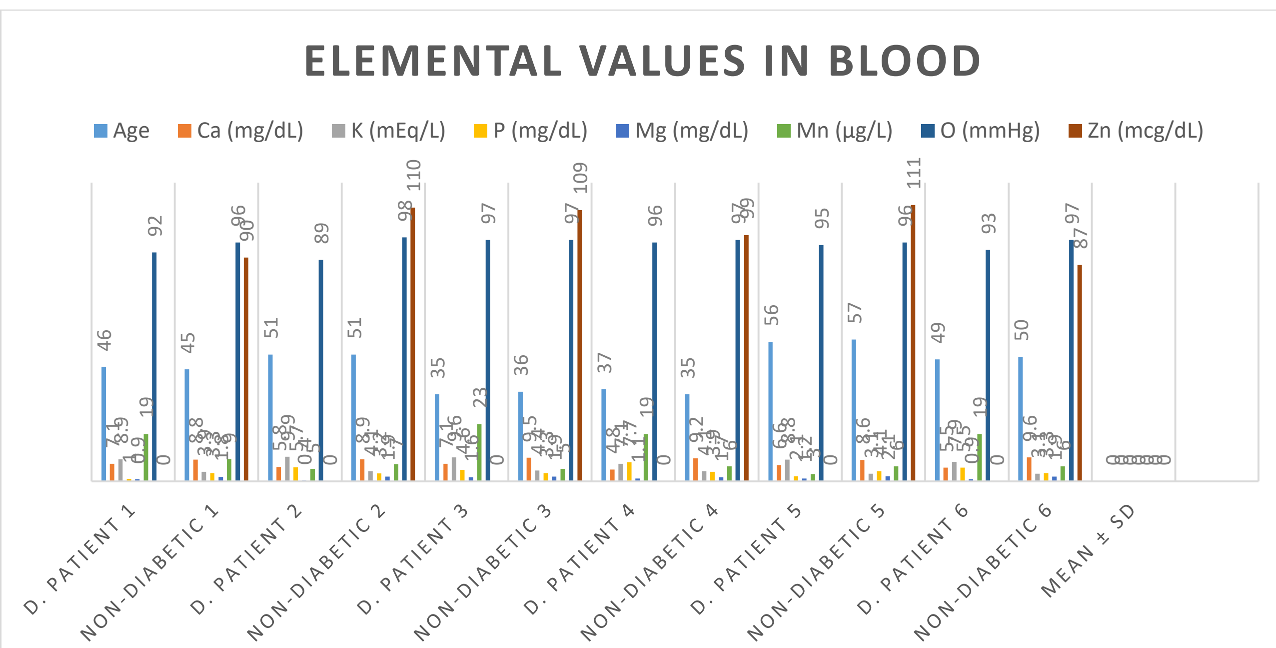

Fig: 1

Table 2. Elemental Values in Hair

Group	Age	Ca (µg/g)	K (µg/g)	P (µg/g)	Mg (µg/g)	Mn (µg/g)	Na (µg/g)	Zn (µg/g)
D. Patient 1	46	156	8	79	20	0.10	15	57
Non-Diabetic 1	45	500	18	145	25	1.20	130	156
D. Patient 2	51	147	4	89	12	0.02	19	42
Non-Diabetic 2	51	565	15	165	35	1.30	123	175
D. Patient 3	35	147	6	78	17	0.04	18	73
Non-Diabetic 3	36	260	16	170	36	0.90	110	174
D. Patient 4	37	178	5	99	13	0.10	14	82
Non-Diabetic 4	35	235	17	179	60	0.80	136	170
D. Patient 5	56	25	4	98	17	0.037	13	33
Non-Diabetic 5	57	210	17	145	70	0.90	139	165
D. Patient 6	49	35	5	87	5	0.045	17	97
Non-Diabetic 6	50	240	19	165	71	1.20	110	162
Mean ± SD		227.08± 163.77	11.83 ± 5.69	122.33 ± 39.64	33.33 ± 22.45	0.58 ± 0.51	70.33 ± 56.78	115.83 ± 55.61

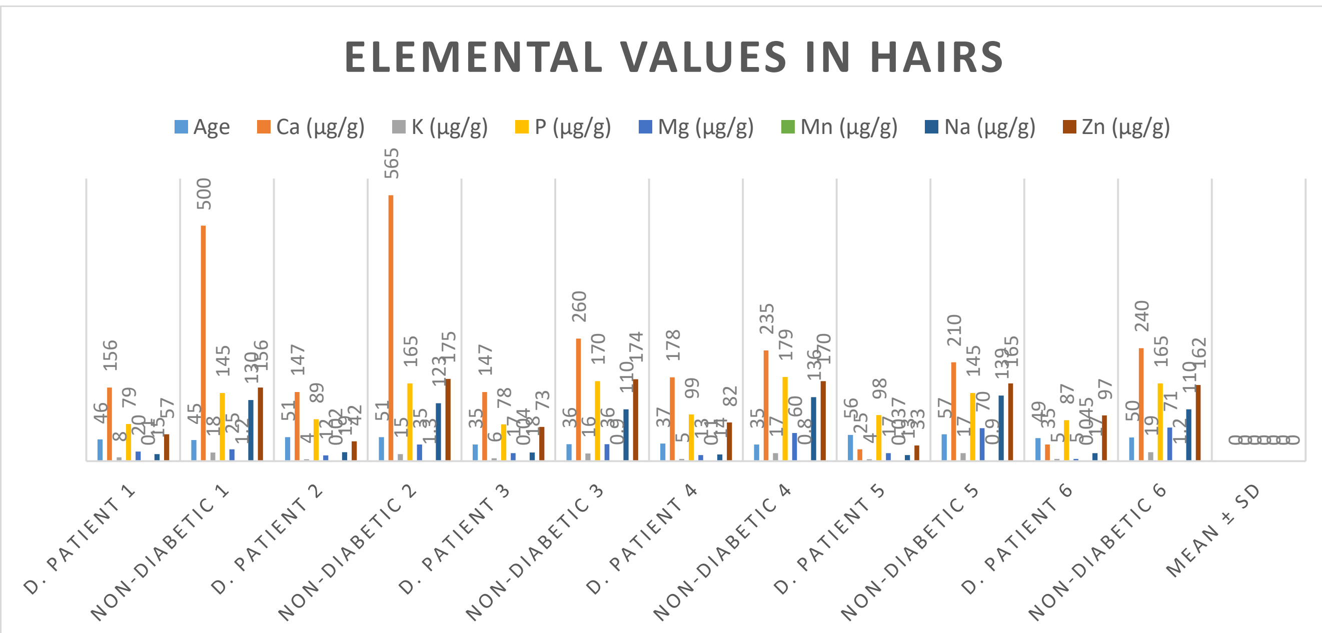

Fig: 2

Table: 3. Elemental Values in Nails

Group	Age	Ca (µg/g)	K (µg/g)	P (µg/g)	Mg (µg/g)	Mn (µg/g)	Na (µg/g)	Zn (µg/g)
D. Patient 1	46	300	199	79	31	0.10	150	56
Non-Diabetic 1	45	1900	1800	654	252	4.80	1300	155
D. Patient 2	51	250	156	89	12	0.20	199	45
Non-Diabetic 2	51	2300	1500	456	152	3.30	1233	110
D. Patient 3	35	298	185	78	17	0.05	178	78
Non-Diabetic 3	36	2600	1600	258	35	0.60	1100	109
D. Patient 4	37	315	178	99	13	0.10	184	87
Non-Diabetic 4	35	2355	1750	754	60	4.30	1356	240
D. Patient 5	56	258	150	98	17	0.04	135	32
Non-Diabetic 5	57	2100	1800	478	98	4.90	1139	111
D. Patient 6	49	352	199	87	5	0.06	147	95
Non-Diabetic 6	50	2400	1700	258	78	3.00	1100	165
Mean ± SD		1244.83 ± 1001.77	886.42 ± 751.76	282.92 ± 247.16	88.33 ± 80.69	1.72 ± 2.06	719.25 ± 558.39	110.25 ± 57.74

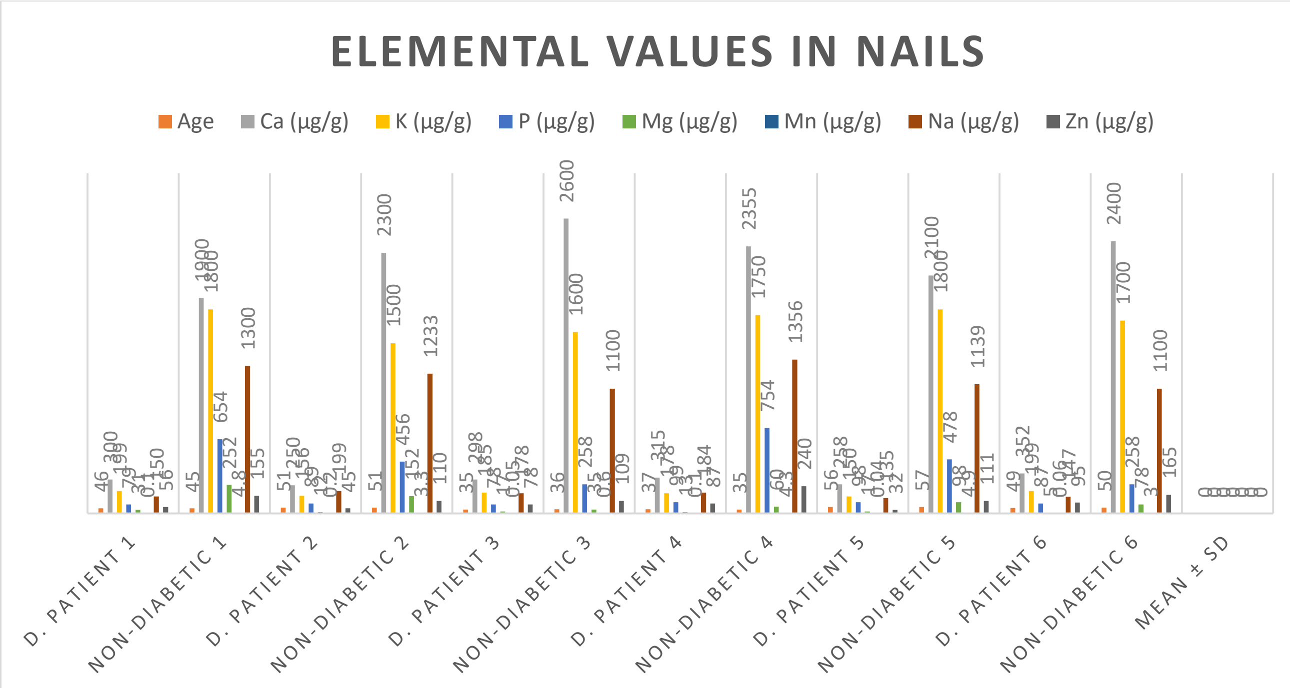

Fig: 3

DISCUSSION

In this study, the elemental composition of blood, hair, and nails was examined in six diabetic and six non-diabetic individuals to determine if trace element imbalances could play a role in the onset and progression of diabetes. The groups were generally similar in terms of age, weight, and history of disease, and sex-related differences were not found for sodium, potassium, zinc, magnesium, or silicon. There was no correlation found between the length of time a person had diabetes and elemental variations, indicating that the imbalances are more likely associated with the disease state itself than with the length of time a patient has been living with the disease. Monitoring of blood pressure indicated greater variability in diabetic patients compared with non-diabetic controls, supporting the established relationship between diabetes and hypertension (Resnick et al., 1994). This study also illustrated how matrices such as blood, nails, and hair can function as biomarkers of long-term metabolic health, while blood more accurately reflects immediate changes, as it can reflect nutritional and metabolic imbalances over a shorter time span. From an immunological perspective, type 1 diabetes occurs when the immune system attacks pancreatic β-cells, causing the body to cease insulin production; environmental triggers, such as viruses, along with a genetic predisposition, are thought to set off the autoimmune cascade (Tripathy and Samanta, 1999; Ashraf et al., 2007). In this context, zinc is particularly relevant as zinc is involved in glucose metabolism, enzymatic regulation and insulin receptor activity; adequate zinc availability enhances insulin secretion and improves glucose tolerance, while zinc deficiency is associated with β-cell vulnerability and impaired metabolic control, and the antioxidant and antiviral properties of zinc may be protective, which could explain why abnormal zinc metabolism has been implicated in the pathogenesis of diabetes and its complications (Barbagallo and Dominguez 2015). In Energy Dispersive Spectroscopy EDS, I found that diabetic patients had significantly higher sodium, magnesium, and lower calcium levels compared to controls, which is consistent with previous studies: magnesium deficiency decreases insulin sensitivity and inhibits insulin release (Sowers et al., 2001), sodium dysregulation can often worsen hypertension, a common comorbidity of diabetes (Resnick et al., 1994), and calcium imbalances can disrupt intracellular insulin signaling and thus worsen glycemic control (Shrivastava et al., 2015). Collectively, these disturbances not only indicate metabolic instability in diabetes, but they also might exacerbate its complications, indicating a potential two-way relationship between trace element deficiency and disease progression. Additionally, comorbid conditions in diabetic patients such as hypertension, cardiovascular disease, chronic kidney disease, hepatitis C, and androgenetic alopecia may also contribute to trace element imbalances, exacerbating the cycle of nutritional deficiency and systemic disease (Barbagallo and Dominguez, 2015). This overlapping pathology underscores the need for a holistic approach to diabetes management that takes into account not only glucose regulation but also micronutrient status. The study was further expanded to analyze the elemental composition of traditional medicinal plants used in the management of diabetes, which contained significant amounts of essential elements, further justifying their ethnomedicinal value, as suggested by Grover et al. (2002). The findings of this study reiterate that elemental disturbances, especially in zinc, magnesium, sodium, and calcium, are not merely coincidental findings but are intimately linked to the pathogenesis and complications of diabetes (Tripathy and Samanta, 1999; Sowers, et al., 2001; Barbagallo and Dominguez, 2015; Shrivastava et al., 2017). And this research also emphasize how treating trace element deficiencies whether through diet, supplements, or the use of medicinal plants can have therapeutic benefits. In addition to enhancing glycemic control, addressing these imbalances may lessen the long-term consequences of diabetes mellitus.

Conclusion: This study also shows that patients with diabetes mellitus have significant changes in trace and essential element concentrations in blood, hair, and nail samples; diabetic patients had lower calcium, magnesium, phosphorus, manganese, sodium, and zinc levels compared with non-diabetic controls, and significantly high potassium levels, with particularly low levels of zinc in the blood, which may be crucially deficient and may have significant implications for insulin function and glucose metabolism. This research support earlier evidence that deficiencies or imbalances in essential trace elements are closely linked to the development, progression, and complications of diabetes, and that because elements like zinc and magnesium are involved in enzymatic regulation, insulin secretion, and cellular metabolism, their deficiency can worsen metabolic dysregulation and contribute to chronic vascular and systemic complications. In addition, this study provides evidence that traditional medicinal plants, like *Chicorium intybus, Syzygium cumini, Citrus decumana, and Withania coagulans,* could be valuable as supplemental therapeutic agents, not only because of their hypoglycemic effects but also because of their contribution of micronutrients. Overall, it is important to conclude that monitoring and correcting trace element imbalances should be considered a part of diabetes management, and that dietary interventions, supplementation, and ethnomedicinal plants can be used in conjunction with conventional therapies to improve glycemic control, reduce the risk of complications, and enhance the quality of life for diabetic patients. This research need further validation with larger partners and further investigation into the therapeutic potential of micronutrient repletion in diabetes care.

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