Assessment of Hematological, Biochemical, and Blood Mineral Parameters in Alkaptonuria Patients in Southern Jordan: A Case-control Study

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ABSTRACT

Background and Aim: Alkaptonuria is rare autosomal recessive disease. It results from a homogentisate 1,2-dioxygenase gene mutation. A major challenge for AKU researchers is the lack of a consistent approach for monitoring illness severity or therapy success. This study investigated blood minerals, hematological and biochemical characteristics, human TNF-alpha, and human Interleukin-10 in the Al-Karak region of southern Jordan.

Method: The study comprised ten people with AKU, ten carriers, and ten controls. Each participant's fasted blood was drawn twice (with and without EDTA) in the morning so that mineral levels, hematological and biochemical parameters, TNF-alpha, and human IL-10 could be analyzed.

Results: AKU patients and carriers had no abnormalities in hematological parameters (HCT, RBC, MCV, WBC, PLT, RDW-CV, and MPV) compared to controls, but HB, MCH, and MCHC were at the lower border of the normal range with no significant change, indicating mild anemia. In addition, female AKU patients and carriers had far higher lymphocyte counts than controls. Biochemical analysis (creatinine, urea, glucose, albumin, total protein, ALT, AST, Alkaline phosphatase, Bilirubin total, and uric acid) and elemental analysis (K, Na, Fe, Mg, Ca, PO4, Zn, Cl, Cu, Ionized Ca++, and Se) were normal regardless of gender. TNF-alpha levels were significantly higher in AKU patients and carriers than controls, but IL-10 levels were significantly lower, regardless of participant group or gender.

Conclusion: The findings of this study could serve as a springboard for further illness research, particularly in rare conditions like AKU.

Key words: Alkaptonuria, AKU, homogentisate, TNF-alpha, elements

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INTRODUCTION

Homogentisate 1,2 dioxygenase (HGD) is a gene on human chromosome 12 that is mutated in this disorder[1]. Because of this, HGD enzyme depletion is common, which in turn reduces tyrosine and phenylalanine catabolism[2]. Ochronosis was diagnosed by biopsies of hip cartilage and intervertebral discs due to the presence of calcification and HGA coloring[3]. Glomerular filtration and active tubular secretion in the kidneys will play a major role in the creation and excretion of the intermediate molecule homogentisic acid[4]. Brownish to black urine is the result of urine being exposed to air or alkalinization[5]. Ochronosis, "the hallmark of AKU"[6], is caused by the oxidation and dimerization of homogentisic acid (HGA) metabolites in cartilages and other connective tissues. Recent redox-proteomic studies have elucidated the molecular basis of ochronosis, leading to suggestions for pharmaceutical intervention[6]. Connective tissues like skin and cartilage accumulate black pigment. After thirty, this coloring is common. Ankylosing spondylitis often causes osteoarthritis in the spine and major joints. Other symptoms of this condition include prostate stones, kidney stones, and cardiac problems[7]. Human homogentisic acid is converted into MAL by HGD during phenylalanine and tyrosine catabolism. Therefore, HGD gene mutations significantly lower homogentisate 1,2-dioxygenase levels and raise homogentisic acid levels. Homogentic acid accumulates slowly in many tissues, including cartilage, despite the kidneys' ability to remove it. In many tissues, excess HGA is oxidized to polymers like melanin[8]. Tissue weakening and darkening, especially of connective tissues like cartilage, come from HGA and its metabolites. This disorder causes tissue to turn dark black or blue[7,9]. Degeneration and destruction of afflicted tissues arise from long-term homogentisic acid accumulation, causing several alkaptonuria symptoms. Most research on this rare disease focuses on treatment, however it turns out that managing symptoms with analgesics, ascorbic acid, and a good diet can prevent it[8].

Joint surgery, heart valve replacements, and organ transplants may be advised as the condition progresses. Alkaptonuria is passed down through the generations as an autosomal recessive characteristic in humans. When an individual inherits a faulty gene from both parents for the same characteristic, several recessive genetic diseases develop. With each pregnancy, 25% of both parents will acquire the defective gene, resulting in an affected child. A 50% probability of having a child with the condition like the parents exists with each pregnancy. No study supports physiotherapy and lifestyle treatment, which are underused. The greatest psychological effects of AKU have also gone unreported[10]. A 50% probability of having a child with the condition like the parents exists with each pregnancy. No study supports physiotherapy and lifestyle treatment, which are underused. Most psychological symptoms in AKU patients are also undiagnosed[10]. Slovakia has the most AKU patients, 208, including 110 children[11, 12]. The prevalence of AKU is 1 in 19 000 in the Dominican Republic and this country[13]. AKU cases have increased significantly in Jordan[14] and India[12], suggesting that the disease's global prevalence may be larger than previously thought.

This study examines whether blood minerals, hematological, biochemical, human TNF-alpha, and human IL-10 are linked to Alkaptonuria disease in Al-Karak province AKU patients. Blood samples from AKU patients, carriers, and healthy controls were tested for minerals, hematological, and biochemical markers to achieve this goal. A field survey, interviews, and medical history analysis selected research participants. The study included AKU patients and carriers who had been diagnosed by clinical examinations and laboratory results (unpublished data) and had not taken antibiotics in three weeks before sample collection. The control samples came from families without AKU cases to ensure their origins were free of mutation.

MATERIAL AND METHODS

Ethical Consideration: This study was approved by the Institutional Ethics Committee (IEC) in Mutah University. The study followed the Declaration of Helsinki (DOH). All participants provided written informed consent.

Study Sample: Using the field survey, interviews, and a review of their medical histories, the study participants (10 AKU patients, 10 AKU carriers, and 10 controls) were selected from the southern Jordan, at coordinates of Latitude 31.1853° North and Longitude 35.7048° East. Patients and carriers with AKU who had previously been diagnosed based on clinical examinations and laboratory results (unpublished data) and who had not taken antibiotics in the three weeks preceding sample collection were selected. To ensure that their roots were completely free of AKU, control samples were taken from families who had never previously reported any AKU cases.

The lineage of AKU family and their ancestors. This study involved a total of 20 people, including 10 AKU patients and 10 AKU carriers. Blood samples have been taken from members of six different families. The carriers who were marked with an asterisk did not take part in the investigation; nonetheless, their information was still included in the pedigree so that it could be used to illustrate the relationships that exist between members of the same family (Figure 1).

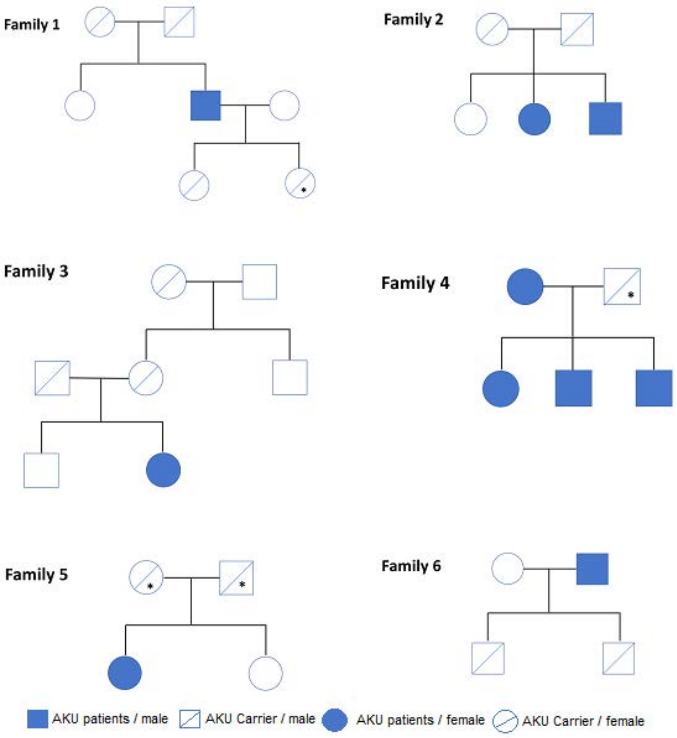

Figure 1: The pedigree of AKU families. 10 AKU patients and 10 AKU carriers were participated in this study. The blood samples have been collected from 6 families. The carriers marked with asterisk did not participate in the study and were mentioned in the pedigree to illustrate the relationship between family members.

Sample Collection: Two blood samples (with and without EDTA) were collected from each AKU patients, AKU carriers, and healthy controls and analyzed for minerals, hematological, and biochemical parameters. Fasting participants' blood samples were obtained in the early morning, kept in an ice box, and delivered to laboratories within one hour. Accordingly, the whole blood samples were used immediately for measuring hematological parameters, meanwhile, the

serum and plasma were obtained from the blood samples and kept at -80 °C for the biochemical heavy metals analysis later.

METHODS

Haematological Parameters: The following haematological parameters were counted and quantified: hemoglobin (HB), hematocrit (HCT), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), platelet (PLT), red blood cell volume distribution width-Coefficient of variation (RDW-CV), mean platelet volume (MPV), and the differential of WBCs (Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Basophils) using Sysmex automated CBC analyser (SYSMEX XE-2100, Japan) after inoculating whole blood sample from an EDTA tube[15].

Biochemical Analysis: The biochemical parameters measured by inoculating serum sample from plan tube into a Cobas c311 Autoanalyzer (Roche Diagnostics, Germany) were creatinine, urea, glucose, albumin, total protein, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, bilirubin, uric acid, K, Na, Mg, Ca, PO_4, Ionized ca^{++}, and Cl [16].

Heavy Metals: The heavy metal (Fe, Zn, and Cu) of the blood were measured by means of Automatic biochemistry analyzer (BA200, BioSystems S.A, Spain) by inoculating serum sample from plane tube. Meanwhile, Se was measured in the blood samples after adding of 5% nitric acid to eliminates the presence of the protein in the samples, thereby excluding the influence of the organic matrix on the result determinations[17], the samples were then analyzed using the Atomic absorption spectroscopy (AA-7000 Schimadzu, Japan).

Human TNF-alpha: An ELISA kit was used to measure TNF-alpha levels in AKU patients, AKU carriers, and control blood. The ab181421 TNF-alpha Human SimpleStep ELISA Kit (which is an in vitro SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit intended for the accurate quantitative detection of TNF-alpha protein in human serum, plasma, and culture medium) was used to measure it according to the manufacturer's procedure[18].

Human Interleukin-10 (IL-10): An ELISA kit was used to determine the amounts of human IL-10 in AKU patients, AKU carriers, and control blood. The ab185986 Human IL-10 SimpleStep ELISA Kit (which is an in vitro SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit developed for the quantitative detection of IL-10 protein in human serum, plasma, and cell culture supernatant samples) was used to measure it according to the manufacturer's procedure[19,20].

Data Analysis: All of the data, which were given as mean ± SD, were statistically assessed using the student's t-test. Basic linear regression was used to investigate the association between the data using the SPSS computer software. A P value of less than 0.05 was used as the lower limit of significance[20-23].

RESULTS AND DISCUSSION

Alkaptonuria (AKU) is thought to be extremely rare, and its cause remains a mystery. Since a genetic mutation is the root cause of this condition, these findings increase the likelihood of identifying the most effective treatment plan. About 40 people in south Jordan have been diagnosed with this illness, and more than 80 people total—men, women, and carriers—have been identified in the Jordanian AKU community[2,7,14,24]. Studies reveal an estimated prevalence of one case for every 250,000-1,000,000 live births, making it challenging to produce standard-appropriate clinical research with correct goals due to the disease's rarity. Researching AKU is difficult because there are no universal criteria for evaluating the severity of disease or the effectiveness of treatment. In order to provide a helpful guide index for doctors and patients for early diagnosis, symptoms, therapies, and diet, more study of this illness is necessary[24].

Haematological Parameters: The results of hematological parameters (HCT, RBC, MCV, WBC, PLT, RDW-CV, and MPV) did not show any significant difference and were all within the normal range, regardless of gender, from AKU patients, AKU carriers, or controls (Table 1). Other parameters such as HB, MCH, and MCHC were within the normal range with no significant change, indicating mild anemia. These findings were consistent with those of Arici and Altun,[25], who discovered no abnormalities in hematological parameters of AKU patients. Tharini et al.[26] discovered that the blood parameters of a 51-year-old man who had AKU since childhood were normal. Davison et al.[27] discovered oxidative haemolysis and a decrease in HB in AKU patients. Jiang et al. [28] reported that routine blood examinations for AKU patients revealed mild anemia and no other abnormalities in the results of hematological laboratory investigations, confirming the current study's findings.

Furthermore, all AKU patients, AKU carriers, and controls had normal differential WBC counts, with the exception of lymphocytes, which showed a significant increase ($P<0.05$) in female of AKU patients and AKU carriers when compared to controls (Table 2). WBCs, also known as leukocytes, are immune system cells that protect the body against infections and foreign bodies[29]. For medical diagnosis, it is crucial to recognize the WBC type and count[30]. When diagnosing low back pain,

Table 1: Hematological parameters in AKU patients, AKU carrier, and control, compared with the normal concentrations, based on the gender

sample ID		HB (g/dl)	HCT (%)	RBC (*10^6/UL)	MCV (FL)	MCH (Pg)	MCHC (g/dl)	WBC (*10^3/UL)	PLT (* 10^3 UL)	RDW-CV (%)	MPV (FL)
AKU-Patients	Male	15.2 ± 0.4	49.8 ± 1.2	5.3 ± 0.1	93.8 ± 2.6	28.5 ± 0.5	30.5 ± 0.8	6.4 ± 1.1	246.8 ± 34.3	13.4 ± 0.8	10.8 ± 1.5
	Female	12.4 ± 0.4	43.4 ± 1.8	4.7 ± 0.2	92.6 ± 2.3	26.5 ± 0.6	28.6 ± 0.4	7.5 ± 1.1	281.4 ± 12.7	14.0 ± 0.5	10.5 ± 0.9
	Bothe male and female	13.8 ± 0.6	46.6 ± 1.5	5.0 ± 0.1	93.2 ± 1.7	27.5 ± 0.5	29.6 ± 0.5	6.9 ± 0.7	264.1 ± 18.2	13.7 ± 0.5	10.6 ± 0.8
AKU-Carrier	Male	14.9 ± 0.5	46.7 ± 1.7	5.3 ± 0.2	90.9 ± 4.3	28.5 ± 0.6	31.5 ± 0.7	6.0 ± 0.3	214.3 ± 16.8	13.3 ± 1.1	12.0 ± 0.3
	Female	13.6 ± 0.7	45.7 ± 3.0	4.7 ± 0.2	97.6 ± 2.8	29.2 ± 0.5	30.0 ± 0.6	6.3 ± 1.0	227.5 ± 15.1	12.5 ± 0.4	11.0 ± 1.0
	Bothe male and female	14.4 ± 0.4	46.3 ± 1.5	5.0 ± 0.2	93.6 ± 2.9	28.8 ± 0.4	30.9 ± 0.5	6.1 ± 0.4	219.6 ± 11.4	13.0 ± 0.7	11.6 ± 0.4
Control	Male	13.2 ± 0.9	39.3 ± 2.1	5.0 ± 0.3	82.2 ± 1.9	26.7 ± 1.2	33.5 ± 0.6	7.6 ± 1.6	289.4 ± 24.0	14.6 ± 0.8	9.9 ± 0.4
	Female	13.6 ± 0.3	40.2 ± 0.6	4.7 ± 0.2	86.4 ± 2.6	28 ± 0.5	33.2 ± 0.5	7.7 ± 0.7	335.3 ± 32.0	13.5 ± 0.3	10.7 ± 0.3
	Bothe male and female	13.3 ± 0.6	39.6 ± 1.3	4.9 ± 0.2	83.7 ± 1.6	27.2 ± 0.8	33.4 ± 0.4	7.6 ± 1.0	306.1 ± 19.5	14.2 ± 0.5	10.2 ± 0.3
Normal range		14-18	42-52	4.7-6.1	80-94	27-31	32-36	4.5-10	150-450	11-15	7-12

Table 2: Differential count of WBCs of AKU patients, AKU carrier, and control, compared with the normal WBCs percentage, based on the gender

Patients ID		Neutrophils	Lymphocytes	Monocytes	Eosinophil	Basophil
AKU-Patients	Male	45.9 ± 3.8	42.5 ± 3.1	8.7 ± 1.8	2.7 ± 0.3	0.8 ± 0.3
	Female	27.6 ± 11.3	57.6 ± 9.1	13.6 ± 3.8	2.5 ± 0.3	0.7 ± 0.1
	Bothe male and female	36.8 ± 6.4	50.0 ± 5.2	11.1 ± 2.1	2.6 ± 0.2	0.7 ± 0.1
AKU-Carrier	Male	54.5 ± 3.8	36.2 ± 3.1	6.9 ± 0.6	1.9 ± 0.4	0.6 ± 0.2
	Female	31.9 ± 12.9	53.7 ± 10.8	13.4 ± 4.1	1.5 ± 0.2	0.5 ± 0.2
	Bothe male and female	45.4 ± 6.4	43.2 ± 5.2	9.5 ± 1.9	1.7 ± 0.2	0.6 ± 0.1
Control	Male	53.9 ± 6.6	35.2 ± 5.5	8.3 ± 1.3	1.8 ± 0.4	0.7 ± 0.1
	Female	51.7 ± 2.8	38.8 ± 3.1	7.3 ± 0.7	1.8 ± 0.3	0.5 ± 0.1
	Bothe male and female	53.1± 4.2	36.5 ± 3.6	7.9 ± 0.9	1.8 ± 0.3	0.6 ± 0.1
Normal range		20-70	15-40	2-10	0.4-4	0.1-1

Table 3: Biochemical parameters of AKU patients, AKU carrier, and control, compared with the normal concentrations, based on the gender

Sample ID		Creatinine (mg/dL)	Urea (mg/dL)	Glucose (mg/dL)	Albumin (g/dL)	Total protein (g/dL)	ALT (U/L)	AST (U/L)	Alkaline phosphatase (U/L)	Bilirubin total (mg/dl)	Uric acid (mg/dl)
AKU-Patients	Male	0.8 ± 0.03	13.02 ± 1.1	99.2 ± 9.2	5.5 ± 0.2	7.4 ± 0.1	32.1 ± 6.6	22.1 ± 1.8	80.4 ± 5.8	0.8 ± 0.04	5.9 ± 0.7
	Female	0.6 ± 0.03	11.5 ± 1.3	102 ± 6.8	4.8 ± 0.3	6.8 ± 0.4	13.8 v 1.5	17.3 ± 1.5	119.2 ± 12.9	0.6 ± 0.1	5.4 ± 0.3
	Bothe male and female	0.7 ± 0.04	12.2 ± 0.8	100.6 ± 5.4	5.2 ± 0.2	7.2 ± 0.2	22.9 ± 4.4	19.7 ± 1.4	99.8 ± 9.3	0.7 ± 0.1	5.6 ± 0.4
AKU-Carrier	Male	0.8 ± 0.1	14.4 ± 1.5	95.8 ± 8.4	5.5 ± 0.3	7.6 ± 0.3	15.6 ± 2.2	21.1 ± 3.8	66 ± 4.1	0.9 ± 0.1	7.1 ± 0.7
	Female	0.7 ± 0.1	18.1 ± 5	77.8 ± 5.8	5.2 ± 0.2	7 ± 0.1	16.4 ± 2.1	17.9 ± 1	114.3 ± 45.7	0.7 ± 0.1	5.6 ± 0.4
	Bothe male and female	0.7 ± 0.1	15.9 ± 2.1	88.6 ± 6.1	5.4 ± 0.2	7.4 ± 0.2	15.9 ± 1.5	19.8 ± 2.3	85.3 ± 18.6	0.8 ± 0.1	6.5 ± 0.5
Control	Male	0.8 ± 0.1	9.5 ± 1.3	101.2 ± 6.4	4.4 ± 0.3	7.6 ± 0.3	15.1 ± 3.9	15.2 ± 1.7	78 ± 12.7	0.78 ± 0.1	5.6 ± 0.5
	Female	0.6 ± 0.04	8 ± 1.3	93.7 ± 2.6	4.5 ± 0.2	7.3 ± 0.5	16.8 ± 3.1	20 ± 2.1	73.5 ± 4.6	0.5 ± 0.1	4.5 ± 0.3
	Bothe male and female	0.7 ± 0.04	8.9 ± 0.9	98.2 ± 4	4.4 ± 0.2	7.5 ± 0.3	15.8 ± 2.5	17.1 ± 1.5	76.2 ± 7.6	0.7 ± 0.1	5.2 ± 0.3
Normal Range		0.4-1.4	5-25	70-110	3.5-6	4-8	0-40	5-40	90-190	0.2-1.2	3.5-7.2

it's important to check the white blood cell count[31]. Lower back and leg pain are common symptoms of AKU patients[32]. Millucci et al.[33] discovered an accumulation of macrophages and lymphocytes in areas of dystrophic calcification and ossification in a 65-year-old woman with AKU, implying inflammation-dependent calcification. The fact that lymphocyte levels are higher in female AKU patients and carriers but not in males is yet unknown. As a result, more research and studies are required, particularly in the absence of previous studies in this field.

Biochemical Parameters: The results of biochemical parameters (creatinine, urea, glucose, albumin, total protein, ALT, AST, Alkaline phosphatase, Bilirubin total, and uric acid) did not show any significance and were all within the normal range, regardless of gender, whether from AKU patients, AKU carriers, or controls, with the exception of Alkaline phosphatase, which showed a reduction in most samples, even in the controls (Table 3). These findings agreed with those of [25], who discovered no abnormalities in AKU patients' biochemical blood parameters. An AKU patient with 51 years of age had normal liver and renal functions tests[26]. For AKU patients, there were no anomalies in the findings of biochemical laboratory tests[28]. In five cases with AKU involving two generations of a single family, all routine blood biochemical and serological assays were normal[34]. Therefore, the current study's blood biochemical parameters were insufficient to be used as a biomarker for AKU disease.

Heavy Metals and Elemental Analysis: The elemental analysis results (K, Na, Fe, Mg, Ca, PO_4, Zn, Cl, Cu, Ionized Ca^{++}, and Se) did not show any significance in any of the tested elements and were all within the normal range, regardless of gender, whether from AKU patients, AKU carriers, or controls, with a slightly higher PO_4 in AKU patients and controls compared to the normal range, though this increase was not significant ($P>0.05$) (Table 4).

The current findings were consistent with those of Curtis et al. (2014), who found that Na, K, Cl, Mg, Ca, PO4, and urea in serum and urine were normal in AKU patients and were unaffected by increases in homogentisic acid (HGA). Patients with AKU should have their antioxidant status evaluated, including trace element measurements of copper, zinc, and selenium, measurement of creatinine to assess renal function, and methaemoglobin measurement for unexplained anemia[35]. Despite this, AKU blood elemental studies are in short supply; consequently, the current study could serve as a foundation for further research into the disease.

Inflammatory level (Human TNF-alpha and Human Interleukin-10 (IL-10)): Figures 2 and 3 demonstrate the serum levels of proinflammatory cytokines and anti-inflammatory, respectively. Impressively, figure 2 shows a statistically significant an increase of the TNF alpha in patient and carrier groups compared to control group, despite no statistically significant change in IL-10 concentration between the groups of patients and carriers. The results demonstrate a statistically significant reduction in IL-10 in the patient and carrier groups when compared to the control group, but no statistically significant difference in IL-10 concentration between the patient and

Table 4: Elemental analysis of AKU patients, AKU carrier, and control, compared with the normal concentrations, based on the gender

		K (mmole/L)	Na (mmole/L)	Fe (µg/dL)	Mg (mg/dL)	Ca (mg/dL)	PO4 (mg/dL)	Zn (µg/dL)	CL (mmole/L)	Cu (µg/dL)	Ionized Ca (mmole/L)	Se (µg/L)
AKU-Patients	Male	4.7 ± 0.1	143.2 ± 1	102.1 ± 15	1.9 ± 0.1	9.9 ± 0.2	6.7 ± 0.4	102 ± 17	105.6 ± 1	88.6 ± 1.5	1.2 ± 0.03	82.2 ± 4.7
	Female	4.3 ± 0.1	137.4 ± 1.4	85.7 ± 10.5	1.9 ± 0.05	9.8 ± 0.2	4.7 ± 0.5	84 ± 16.1	107.4 ± 0.7	103.8 ± 9.4	1.2 ± 0.03	71.4 ± 5.4
	Bothe male and female	4.5 ± 0.1	140.3 ± 1.3	93.9 ± 9	1.9 ± 0.03	9.9 ± 0.1	5.7 ± 0.4	93 ± 11.5	106.5 ± 0.7	96.2 ± 5.1	1.2 ± 0.02	76.8 ± 3.8
AKU-Carrier	Male	4.5 ± 0.2	140.7 ± 0.8	92.6 ± 13.3	1.9 ± 0.1	10.1 ± 0.1	4.1 ± 0.1	66.2 ± 7	106.3 ± 1.1	85.7 ± 1.1	1.2 ± 0.01	74.2 ± 5.8
	Female	4.3 ± 0.2	139 ± 2	77.9 ± 5.7	1.7 ± 0.05	9.8 ± 0.3	3.7 ± 0.1	55.5 ± 4	105.3 ± 1.8	88 ± 2.6	1.2 ± 0.04	80.5 ± 7.5
	Bothe male and female	4.4 ± 0.1	140 ± 0.9	86.7 ± 8.3	1.8 ± 0.04	10 ± 0.1	4 ± 0.1	61.9 ± 4.6	105.9 ± 0.9	86.6 ± 1.2	1.2 ± 0.02	76.7 ± 4.4
control	Male	4.5 ± 0.1	137.9 ± 0.6	93.7 ± 15.3	2 ± 0.1	9.4 ± 0.3	5.6 ± 0.3	82.6 ± 13	105.6 ± 1.6	100.9 ± 7.5	1.2 ± 0.02	96.1 ± 2.1
	Female	4.3 ± 0.1	140.3 ± 1	88.1 ± 12.8	2 ± 0.1	9.8 ± 0.3	5.3 ± 0.7	79.1 ± 13	105.5 ± 2.3	93.3 ± 5.4	1.2 ± 0.1	91.3 ± 1.5
	Bothe male and female	4.4 ± 0.1	138.7 ± 0.6	91.6 ± 10.4	2 ± 0.04	9.6 ± 0.2	5.5 ± 0.3	81.3 ± 9	105.5 ± 1.2	98.1 ± 5.1	1.2 ± 0.02	94.4 ± 1.6
Normal Range		3.5-5.5	135-155	60-170	1.7-2.4	8.5-10.5	3-4.5	46-150	95-111	80-155	1.2-1.4	70-130

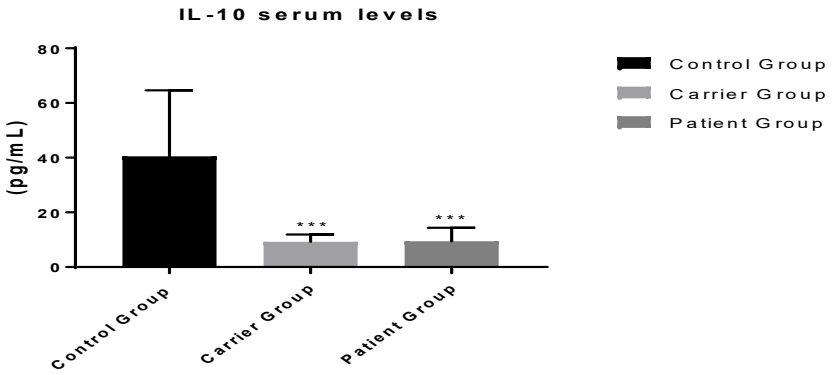

Figure 2: The serum levels of IL-10 between the three study groups. The results represent the concentration of IL-10. The results are expressed as means ± SD (n = 10 independent replicates). ***p<0.001 compared to control group. The results were statistically analyzed using one-way ANOVA.

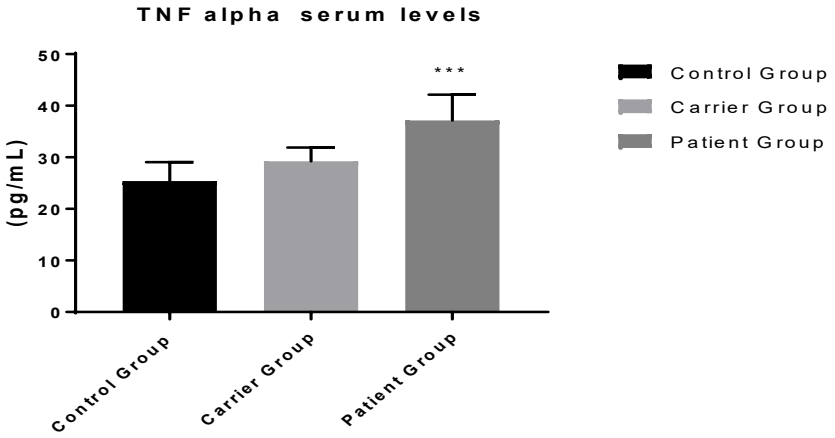

Figure 3: The serum levels of TNF alpha between the three study groups. The results represent the concentration of TNF alpha. The results are expressed as means ± SD (n = 10 independent replicates). *** p<0.001 compared to control group. The results were statistically analyzed using one-way ANOVA.

carrier groups (Figure 3). Yuan et al.[36] found a favorable correlation between IL-10 and urine protein levels. AKU Patients are at risk of protein depletion due to a "perfect storm" of risk factors, including historical, shaky evidence-based recommendations to cut total protein intake[37]. TNF-α is a critical therapeutic target in many chronic inflammatory diseases[38–40]. TNF-α appears to be a key mediator of this connection, linking inflammation in patients with alkaptonuria and its carriers. Such outcomes might, at least in part, be connected to the gene(s) in charge of causing AKU diseases and AKU carriers.

Alkaptonuria is a complicated inflammatory multisystemic illness that affects several different organs[41], including the heart[42], kidney[43], liver[44], and lung[45]. Several studies have found that persistent accumulation of homogentisate1,2-dioxygenase in Alkaptonuria can trigger a number of events that increase inflammatory responses by increasing the secretion of proinflammatory cytokines while lowering the release of anti-inflammatory cytokines[46,47]. As a result of our findings, we can conclude that controlling the underlying inflammatory illness can lead to disease regression.

CONCLUSION

Males and females are likewise affected by alkaptonuria. The disease is, however, regarded rare or extremely rare, and the origin of Alkapton sickness is unknown. In any case, these findings expand the possibilities for discovering the best treatment strategy, especially since this type of disease is caused by a genetic mutation. In Jordan, there are about 40 cases of this ailment in south Jordan, and over 80 cases have been reported in the Jordanian AKU society, including both males and females, as well as disease carriers. This characteristic is regarded as one of the difficulties to statistically investigating the disease and makes producing standard-appropriate clinical research with proper goals difficult when studies suggest an estimated occurrence of one case out of every 250,000-1,000,000 live births. As a result, one of the most significant challenges in researching AKU is the lack of a consistent method for assessing illness severity or therapy response. Therefore, more research into this illness is recommended in order to develop a useful guide index for physicians and patients regarding early diagnosis, symptoms, treatments, and nutrition.

Authorship Contribution: The study's conception and design were handled by AMK, NRM, RKA, RSD, and AMAT, while NRM, RKA, and IAS and AHAT were in charge of data collection. The data were processed and interpreted by AMK, NRM, RKA, RSD, and AT. The manuscript was written by all authors, who also gave it thorough revisions. The version that was submitted to this journal was also read by all authors, who gave it their final approval.

Ethical Consideration: The research followed the guidelines laid out in the DOH (Declaration of Helsinki). All regulations regarding the treatment of human subjects in medical research were strictly followed. The privacy and autonomy of the research participants were respected at all times.

Potential Conflict of Interest: None

Competing Interest: None

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