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Investigation of the Association Between Vitamin D and Uric Acid Levels in a Potassium Oxonate-Induced Gouty Rat Model

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Abstract

The major objective of the present study has been to investigate a correlation between an elevated level of vitamin D and serum level of uric acid in a gouty rat model created by potassium oxonate. The experiment involves examining the impact of vitamin D3 on serum uric acid in hyperuricemic rats created with potassium oxonate. Animal study (rats) was randomly divided into four groups (n = 6). Potassium oxonate (uricase enzyme inhibitor) was injected intraperitoneally (i.p) at a dosage of 250 mg/kg to the rats of groups (B-D-Z) twice a week. Group (A) fed with food and water without treatment and served as a negative control group. Group (B) was orally administered with 0.5 ml of Olive Oil (vehicle) once weekly and served as a positive control group. Animals of the group (D) received Vitamin D3 in a dose (715 I.U./kg) orally once weekly. Group (Z) rats were treated orally with standard drug(allopurinol) (5 mg/kg) daily. Animals of each group were sacrificed and blood was collected and serum was separated for biochemical analysis of serum uric acid (SUA), serum creatinine (SCr), blood urea, AST (Aspartate transaminase), ALT (Alanine transaminase) and ALP (Alkaline phosphatase) levels, Serum levels of xanthine oxidase and Serum level of vitamin D were assay. The study's findings revealed a highly significant rise in blood uric acid and xanthine oxide in hyperuricemic rats administered vitamin D. There are non-significant differences in liver functions (ALT, AST, and AlkP) and kidney functions (Urea and SCr) have been seen for all three groups (B, D, and Z) as compared with the negative control group. This study revealed that uric acid levels in the blood showed a positive correlation with vitamin D concentration.

Keywords: *Vitamin D, Uric acid, Xanthine oxidase, Renal function, Liver function, Hyperuricemia*

1. Introduction

Hyperuricemia is a frequent pathological condition marked by an elevation of the blood level of uric acid, resulting in the collection of urate crystals in soft tissues such as the joints and kidneys. Hyperuricemia is a major responsible factor for a variety of illnesses, including gout, uric acid nephrolithiasis, cardiovascular disease, and renal disease (1). Some studies have shown that approximately 15-20% of people have gout but to varying degrees. Adult men have a maximum normal uric acid level of 7.7 mg/dL while adult women have a maximum normal level of 6.6 mg/dL (2). Uric acid (UA) is produced by metabolizing purines as an end product in humans due to uricase deficiency (3). The enzyme xanthine oxidase (XOD) is responsible for producing uric acid by converting hypoxanthine to xanthine, which is subsequently transformed into UA (4, 5).

Hyperuricemia develops in individuals based on excessive uric acid production and/or underexcretion (UA). Consumption of foods rich in purines in large quantities and a genetic enzymes deficiency can trigger the development of hyperuricemia (6).

Urate-lowering drugs in the market can be classified into different categories according to their action mechanism: Xanthine oxidase inhibitors (such as zyloric), uricosuric drugs (such as probenecid), and uricase injection (such as pegloticase). Though, repeated use of allopurinol as a xanthine oxidase inhibitor may cause Allopurinol Hypersensitivity Syndrome (AHS), which can cause serious side effects such as skin rash and GI problems, as well as potentially fatal allergic responses (7, 8).

Vitamin D_3 is a lipid-soluble vitamin and is

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important for the health maintenance of humans. It can be gained in ergocalciferol (D_2) shape from plants and fungi and as a form of cholecalciferol (D_3) mainly from animal products. In addition, the body can produce it through the exposure of the skin to UV light (under the action of ultraviolet light at 7-dehydrocholesterol present in the skin). However, keratinocytes in the skin are the main source of Vitamin D and to a lesser extent from dietary and supplemental sources (9, 10)**.** Vitamin D_3 also can be obtained from the diet and this way has special importance for people who have limited exposure to sunlight.

Vitamin D has a wide range of roles include the development and maintenance of bone tissue, the maintenance of homeostasis of calcium and phosphorous, and it is also engaged in many different vital cellular processes like cell differentiation and proliferation, hormone secretion (for example insulin), role in the immune system, and several chronic diseases such as obesity (10)**.**

Previous studies have shown that increased plasma levels of 1,25(OH)2-vitamin D_3 reduced plasma uric acid levels by 42%. As a result, blood levels of 1,25(OH)2D_3 have been observed to fall in gout patients (11). Hyperuricemia is related to low levels of 1,25-(OH)2D_3 by inhibiting 1-α hydroxylase activity (12). Vitamin D deficiency has been correlated with higher uric acid levels in Chinese postmenopausal women in Han City, according to multiple studies (13). People with both vitamin D insufficiency and D deficiency exhibited greater amounts of uric acid in their blood when compared to normal levels, according to Nipith Charoenngam *et al*, 2020 (14).

Conversely, higher SUA levels have been linked to decreased vitamin D levels in those with chronic kidney disease and type 2 diabetes, according to previous studies. In another investigation, serum uric acid and 25-(OH) D were shown to have a favorable association in east Chinese individuals (15). Hyperuricemia can be indicated by high levels of 25-(OH) D in the blood (9).

Other vitamins also affect blood uric acid as vitamin C (Ascorbic acid) has a negative relationship to serum uric acid level, and vitamin C supplementation significantly reduces SUA (16). It shows anti-hyperuricemia activity through uricosuric effect in an oxidative damage and kidney injury-induced hyperuricemic rat model (17). A negative relationship has been noticed between vitamin E consumption and hyperuricemia in adults, especially among males aged ≥ 60-year-old participants (18), and it has anti-hyperuricemia activity against rats induced by hyperuricemia (19).

Woo-Joo Choi *et al*, 2012 (20) demonstrated that a high intake of vitamin A supplements may donate to the large prevalence of hyperuricemic patients in the United States, while intake of carotene leads to more protection against hyperuricemia. Other research found that folic acid or vitamin B_{12} consumption was inversely associated with the incidence of hyperuricemia (21).

As a result, there are numerous connections between vitamin D and SUA, as previously indicated. The purpose of the present study was to inquire into the relationship between serum uric acid and 1,25-(OH)2D_3.

2. Experimental

2.1 Materials

Sigma-Aldrich Co., St. Louis, CA, USA provided potassium oxonate and allopurinol (Zyloric). Vitamin D_3 (Diabase) was purchased from Newbridge Pharmaceuticals in the United Kingdom.

2.2 Animals

This study included the use of twenty-four Swiss adult male rats, weighing about (180-200 grams), supplied from the animal house at the Pharmacy College, University of Basrah. The animals were separated into four groups, each with a different set of characteristics (n = 6). For a week, rats were kept in separate plastic cages and put in the animal room under regulated conditions of 22 ± 4 °C, 30 ± 15 percent humidity, and a 12-hour dark/12-hour light cycle. Throughout the trial, the animals were given free access to standard chow and water.

2.3 Methods

2.3.1 Drug administration

Allopurinol (Zyloric), 5 mg/kg was dissolved with 0.5 L of distilled water (D.W). Potassium oxonate (PO), 0.25 g/ kg was dissolved in the warm normal saline solution. Vitamin D_3 715 I.U./kg (50000 I.U./70 kg) was dissolved in olive oil. All solutions were prepared immediately before the experiments (22).

2.3.2 Induction of hyperuricemia

Experimentally, hyperuricemia in the experimental animals (rats) was induced by intraperitoneal (i.p.) injection of potassium oxonate (uricase inhibitor) in a

dose (0.25 g/kg) twice a week for 2 months (22).

2.3.3 Experimental study design

The influence of vitamin D_3 on blood uric acid levels was demonstrated using a hyperuricemic rat model triggered by potassium oxonate with slight adjustments (Hameed & Ramadhan, 2018) (23). Animals were fasted for two hours before receiving medicines and vehicles by withdrawing food and water. The experimental rats were randomly categorized into four groups (n = 6). Every three days during the experiment period (two months), a uricase inhibitor (potassium oxonate) was given as an intraperitoneal (i.p.) injection at a dose of 0.25 g/kg into the animals' groups (B-D-Z). Group (A) animals were fed food and water without treatment to be served as a negative control. Group (B) rats were orally administered with 0.5 ml of Olive Oil (vehicle) by oral gavage once weekly of the experiment and served as a positive control group. Animals of the group (D) were orally administered Vitamin D_3 in a dose of 715 I.U./kg (50000 I.U./70 kg) once a week. Animals of the (Z) group were treated orally with the standard drug, allopurinol (5 mg/kg) daily during the experiment period.

2.3.4 Collection of blood samples

After the end of the two-month study period, the rats were fasted overnight and then anesthetized with chloroform inhalation. Whole blood samples from experimental animals were collected by cardiac puncture, then to be put in gel tubes. To obtain serum, blood samples were allowed to coagulate at ambient temperature for a half-hour before being centrifuged for 10 minutes at 4000 rpm. Finally, sera were kept at a temperature of -20 °C until biochemical parameters were measured.

2.3.5 Biochemical parameters Assays

Serum uric acid (SUA), serum creatinine (SCr), blood urea, AST (aspartate transaminase), ALT (alanine transaminase), and ALP (alkaline phosphatase) levels were measured in the serum using standard diagnostic kits utilizing enzymatic-colorimetric approaches (BioLab., France). Xanthine oxidase (XO) levels in the blood were determined using BT LAB enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, China). Serum level of vitamin D was determined in serum by using an automatic immunoassay analyzer (ARCHITECT plus i1000SR, Abbott, U.S.A.).

2.3.6 Statistical analysis

The data of all trials in this study are presented as mean ± standard deviation. ANOVA was used for statistical analysis, which was followed by Dennett's t-test. The probability (*P*) values less than 0.05 were considered statistically significant.

3. Results

3.1 Xanthine oxidase

As could be seen in the results in Table (1), It was noticed that there was a highly significant ($P<0.01$) increase in xanthine oxidase for group B (positive control group, treated with olive oil only) compared with a negative control in each blood sample. While in the group (D) when compared with negative control there was a very high significant ($P<0.001$) increase in the xanthine oxidase, and there was a significant ($P<0.05$) increase in xanthine oxidase in the group (Z) compared with a negative control in each blood sample.

3.2 Serum uric acid

In comparison to the healthy animals' group, intraperitoneal injection of potassium oxonate at a dosage of 0.25 g/kg significantly increased SUA levels in rats, as indicated in Table (1). In contrast to the negative control animals, SUA increased significantly ($p<0.001$) in both groups (B and D). In hyperuricemic rats, treatment of a conventional XO inhibitor (allopurinol at a dosage of 5 mg/kg, p.o.) caused a very significant ($p<0.001$) reduction in SUA.

Table I: Biochemical petameters of (A, B, D, and Z) rats' groups.

Group	Xanthine (µg/ml)	UA (mg/dl)	ALT (UI/L)	AST (UI/L)	AlkP (UI/L)	Urea (mg/dl)	SCr (mg/dl)	Vit.D (pg/ml)
A	20	1.82	42.6	126.5	210	26.5	0.42	16.5
B	30 **	3.39 ***	42.3	131.6	222	25	0.39	23.6 ***
D	46.6 ***	4.11 ***	41.4	130.6	201	28.4	0.41	32.4 ***
Z	24.6 *	0.62 ***	41.2	133.8	241	22.8	0.41	17.2

*$p<0.05$, **$p<0.01$, ***$p<0.001$

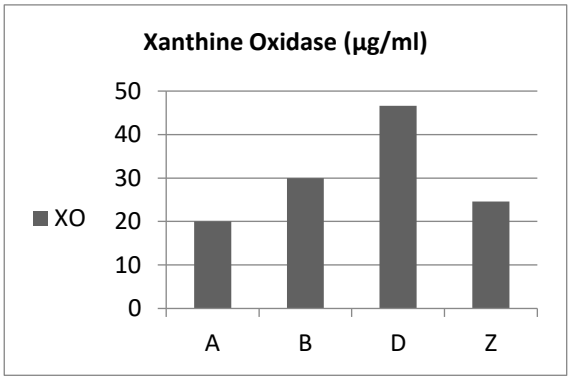

Figure 1: Xanthine oxidase levels(µg/ml) in the serum of rats for groups (A, B, D, Z).

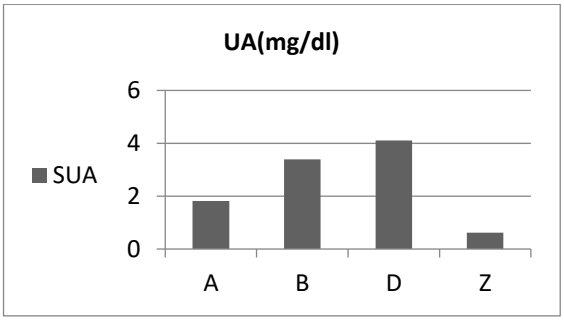

Figure 2: Uric acid levels (mg/dl) in the serum of rats for groups (A, B, D, Z).

3.3 Vitamin D

As we saw in table (1) there is a very high significant (P<0.001) increase in vitamin D level for the group (D) and (B) in comparison with a negative control in each blood sample.

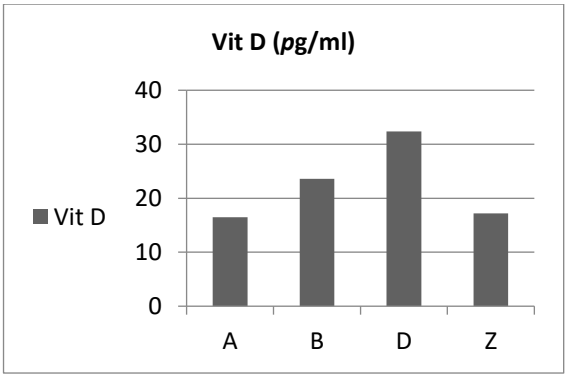

Figure 3: Vitamin D3 levels (pg/ml) in the serum of rats for groups (A, B, D, Z).

3.4 Liver and kidney functions

As clarified in Table (1), no significant alterations in liver function (ALT, AST, and AlkP) and kidney function (Urea and SCr) were observed in the three groups (B, D, and Z) as compared with the animals of negative group.

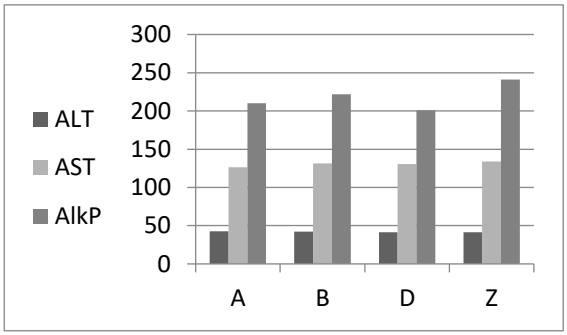

Figure 4: ALT, AST and AlkP levels (UI/L) in the serum of rats for groups (A, B, D, Z).

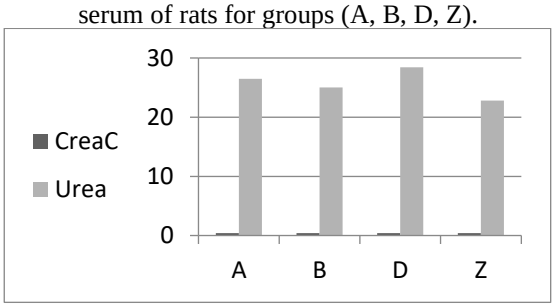

Figure 5: Blood urea and serum Creatinine levels (mg/dl) in the rats for groups (A, B, D, Z).

4. Discussion

The mechanism of hyperuricemia was studied in this work by using hyperuricemic rats which are induced with potassium oxonate as an acceptable experimental model. Both the conversion of hypoxanthine to xanthine and the conversion of xanthine to uric acid are catalyzed by the XOD enzyme (24).

The pathogenesis of hyperuricemia may be studied using potassium oxonate-induced hyperuricemic rats as an animal model. The intraperitoneal administration of (250 mg/kg) into rats twice weekly for two months elevated serum UA and decreased uric acid in the urine through two mechanisms: first, a rise in blood xanthine oxidase levels (22), and second, inhibition of the uricase enzyme (23).

Because of its low cost and quick action, potassium oxonate is a particularly effective uricase inhibitor in animal models of hyperuricemia. In this study, rats administered potassium oxonate had a much higher (p <0.001) rise in the blood level of urate when related to the animals' negative group. Hyperuricemia has been treated with allopurinol, an XOD inhibitor. The daily administration of allopurinol (5 mg/kg) throughout the length of the study resulted in a substantial (P<0.001) decrease in the SUA level. The inhibition of XO caused a substantial decrease in the level of SUA (23).

In contrast, a weekly oral treatment of vitamin D_3 at a dosage of 715 I.U./kg (50000 I.U./70 kg) resulted in a very important ($P<0.001$) increase in serum UA levels of (D) group as equated to the rats of the negative group due to an increase in xanthine oxidase levels in the blood (9).

High SUA can cause a rise in 25(OH)D concentration. Vitamin (D) augmented blood UA levels in vitamin D-treated rats, perhaps because hyperuricemia inhibits 1-hydroxylase, limiting the converting of the 25(OH)D form of vitamin D into calcitriol [1,25(OH)2D]. Vitamin D has indeed been associated with hyperuricemia in some studies, with 25-(OH) D levels being greater in hyperuricemic patients than in non-hyperuricemic people (9). Furthermore, a reduction in SUA was revealed to be one of the predictors of hypovitaminosis D in another research (15). However, numerous earlier investigations have provided a correlation between HUA and vitamin D insufficiency (13, 27, 28), demonstrating a complex relationship with both vitamin D level and SUA (9).

In our findings, we observed that an elevated level of vitamin D (hypervitaminosis) did not affect liver function and also had no effect on kidney function (by testing for liver enzymes, blood urea levels, and creatinine).

Despite the lack of a clear causal relationship between SUA and vitamin D, we must be cautious of the dangers of excessive vitamin D use. To establish more about the effect of vitamin D supplements on blood uric acid levels, further clinical trials are needed.

5. Conclusions

In a rat model of oxonate-induced hyperuricemia, the influence of vitamin D on uric acid was examined, and a substantial elevation of both sera (uric acid and xanthine oxidase) was seen without affecting liver or kidney function. As a result, this research suggests that uric acid levels in the blood have a favorable relationship with vitamin D levels.

5. Conflicts of interest

There are no conflicts to declare

6. Acknowledgments

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