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Effect of Xanthine Oxidase Inhibition on Immunity Modulation in Human

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Abstract: Xanthine oxidase (XO) is a ubiquitous complex cytosolic molybdoflavoprotein which controls the rate limiting step of purine catabolism by converting xanthine to uric acid. It is known that optimum concentrations of uric acid (UA) and reactive oxygen species (ROS) are necessary for normal functioning of the body. Aim: This study is conducted to clarify the impact of xanthinoxidase (XO) inhibition on immunity function in human. Materials & Methods: Patients with renal stone were enrolled in this study. Patients took xanthine oxidase inhibitor (allopurinol) 100 mg/day for 6 months to reduce UA level, and the immune function is estimated by measuring some immunological parameters (IgG, IgA, complement C3 and C4). Blood samples were collected from patients before and after therapy. Autoanalyzer was used to measure the level of uric acid, IgG, IgA, complement C3 and C4. Results: Serum uric acid was significantly lowered after the treatment with allopurinol. Both IgG and IgA were elevated significantly due to allopurinol intake. However, complement C3 and C4 have not been changed after the same period of treatment. Conclusion: Long term inhibition of xanthine oxidase may affect the immunity by modulating immunological parameters in patients with renal stone.

Keywords: Complements, Immunity modulation, Immunoglobulins, Xanthine oxidase inhibition.

Abbreviations

XO, xanthine oxidase; UA, uric acid; ROS; reactive oxygen species.

1. INTRODUCTION

Xanthine oxidase (OX) is a dimeric metalloflavoprotein enzyme widely distributed among species including human, which facilitates the catabolism of purines. Firstly, purines, adenosine monophosphate (AMP) and guanine monophosphate (GMP) are converted into xanthine or hypoxanthine. Then, XO enzyme causes further oxidation and breakdown of xanthine and hypoxanthine into uric acid (UA) [1]. As there is a continual degradation and renewal of purines, this XO reaction is critical in cell turnover and vital to mammalian cells [2]. In addition, XO is a physiological source of reactive oxygen species (ROS) (e.g.,

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superoxide ion, and hydrogen peroxide) which are considered as important second messengers for several cell-signalling pathways in the body [3].

Optimal uric acid concentration is critical for many body functions. The overproduction and/or underexcretion of uric acid causes a pathological condition called hyperuricaemia [1], [4]. High level of UA has a negative impact on many systems in the body including musculoskeletal and renal systems. Gout is a chronic, progressive musculoskeletal disorder characterized by a high level of plasma uric acid which leads eventually to deposition of monosodium UA crystals in different joints and tendons [5]. Hyperuricemia can cause acute kidney injury [6] and is considered as a risk factor for chronic kidney diseases [7]. Renal stones are among the most common urinary tract problems, and UA stone is considered as the second most-common cause of kidney stones [8]. XO enzyme is a well-established target of drugs used for treatment of hyperuricemia, gout and renal stones [9], [10].

Level of immunity is essential for life as the immune system provides a safe space to perform the normal functions of life [11]. Different types of cells are responsible for immunity status including immunoglobulins and complements. For instances, IgG, which accounts for about 75% of the total immunoglobulins in the plasma, protects the internal organs from the invading microorganisms, and considered as the main neutralizing antibody for toxins in tissues due to its easy diffusion throughout extracellular fluid [12]; whilst IgA is the principal antibody that protects the mucosal surface of gastrointestinal, respiratory, and genitourinary tract from invading pathogens [13]. Complements have crucial roles in both innate and adaptive immune responses. When complements are activated due to presence of invading microorganism series of proteolytic cascades are proceeded to end with lysis of the pathogens in addition to proinflammatory bioactive factors are generated to stimulate the classical inflammatory response [14]. Immunity can be modulated by many factors including host and environmental factors (reviewed in [15], [16]).

As XO pathway is considered as an important pathway for production of ROS and the high oxidative stress has a significant impact on immunity, XO pathway may play a crucial role in modulating immunity. Moreover, a recent review highlighted how the XO Pathway is regarded as a major source of hydrogen peroxide and superoxide, and how these ROS can elicit significant bacteriostatic and bactericidal effects [17]. In addition to the various essential biological effects of ROS, they can elicit different pathologies including inflammation and impaired immune response [18]. However, the mechanisms of immunity modulation by xanthine oxidase and UA have been a subject of increasing interest for many researchers and it is still a matter of debate. The present study is conducted to investigate the effect of long-inhibition of XO on the specific parameters of the immunity in human.

2. MATERIALS AND METHODS

Eight patients with renal stone were treated with allopurinol; at an optimal therapeutic dose of 300mg/day for at least 6 months. Patients information (age, sex, and BMI) are presented in table 1 (see below). The record of patients also included an agreement consent forms for participation in the study. Patients with any sort of chronic clinical illness were excluded from study include pregnant and lactating women. Blood samples were withdrawn from

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individual patients (before and after allopurinol therapy) and serum collected and frozen to be ready for further processing.

The levels of uric acids were measured colorimetrically before and after therapy, to confirm that allopurinol reduces uric acid properly. To do so, serum samples were treated by reaction mixture provided by the kit, the mixture with serum were incubated shortly (half-hour) to ensure that the reaction completed. The samples colors were then measured by spectrophotometer. The principle of assay is based on uric acid oxidation to allantoin and CO_2, this reaction was carried out by phosphotungistic acid reagent in alkaline solution followed by a conversion of the phosphotungistate to tungsten blue; to be measured spectrophotometrically (O.D. 710nm) (Fig 1)

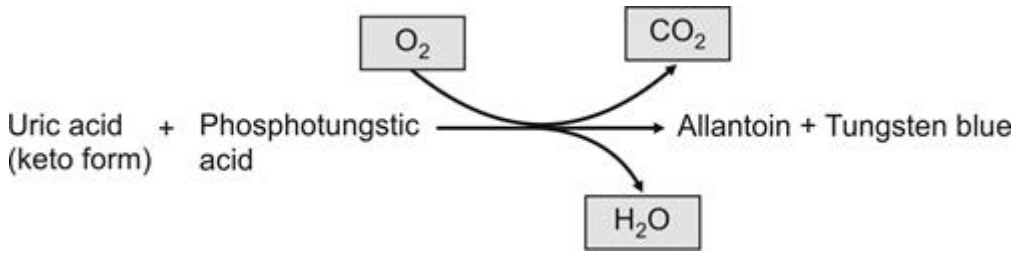

Figure 1. Principle used for measurement of uric acid.

The measurement of proteomic components (complements and immunoglobulin) were performed using sandwiched ELISA technique. The procedure involves coating 96-wellplates with capture antibodies specified for target protein. The plates were then foiled and incubated at room temperature overnight. In the next day morning, the plates were thoroughly and carefully washed four times with a washing buffer and blocked by blocking buffer. A hundred millilitres of plasma samples and diluted standards were then loaded into the wells with a micropipette and foiled and incubated for 2 hours to be followed by gentle washing with washing buffer. Immediately following the washing step, a 100 ml of previously diluted detection antibodies were loaded into the wells and foiled and incubated at room temperature for 2 hours. This step was followed by washing and subsequently the wells were exposed to horseradish peroxidase enzyme (avidine), the enzymatic reaction has to continue for half an hour and then to be terminated by stop solution producing faint yellow colour solution to be measured at OD 450 nm. The colour intensity of which is reciprocal to the quantified protein detected. The concentrations of protein were then interpolated from the calibration curve of standard solution.

TABLE I. BIOCHEMICAL AND DEMOGRAPHIC CHARACTERISTICS OF THE STUDIED GROUPS.

	Allopurinol Therapy (n=8)	
Parameters	Before	After
Age (years)	39.41±3.8	-------
BMI (kg/m^2)	28.2±3	28.5±2.8
Duration of treatment	6 months	-------

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*p<0.05, BMI=body mass index, kg=kilogram, m^2=square meter

A. Statistical Analysis

Data were expressed as the mean ± standard deviation. Comparisons between the investigated parameters for allopurinol-treated patients before and after therapy were conducted using the t-test. $p<0.05$ was considered a statistically significant difference. Statistical results were obtained using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

3. RESULTS

A. Effect of XO Inhibition on UA

Serum uric acid is firstly measured to check the extent of activity of XO enzyme and investigate the impact of its inhibition in patients with renal stone. Serum UA has been reduced significantly after 6 months treatment with XO inhibitor allopurinol (Fig 2).

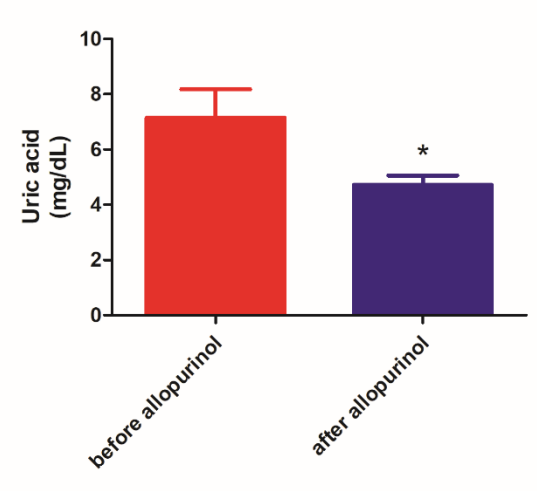

Figure 2: Effect of XO inhibition on UA plasma level. Data are expressed as mean ± SEM for comparison of UA level before and after treatment with allopurinol for 6 months. * indicates P < 0.05, Student's one-tailed, paired t-test.

B. Effect of XO Inhibition on Plasma Levels of IgG and IgA in Renal Stone Patients.

In order to check the effect of XO inhibition on immunity modulation in renal stone patients, IgG and IgA are measured. The plasma levels of the two immunoglobulins are significantly elevated after 6 months of allopurinol treatment (Fig 3 A,B).

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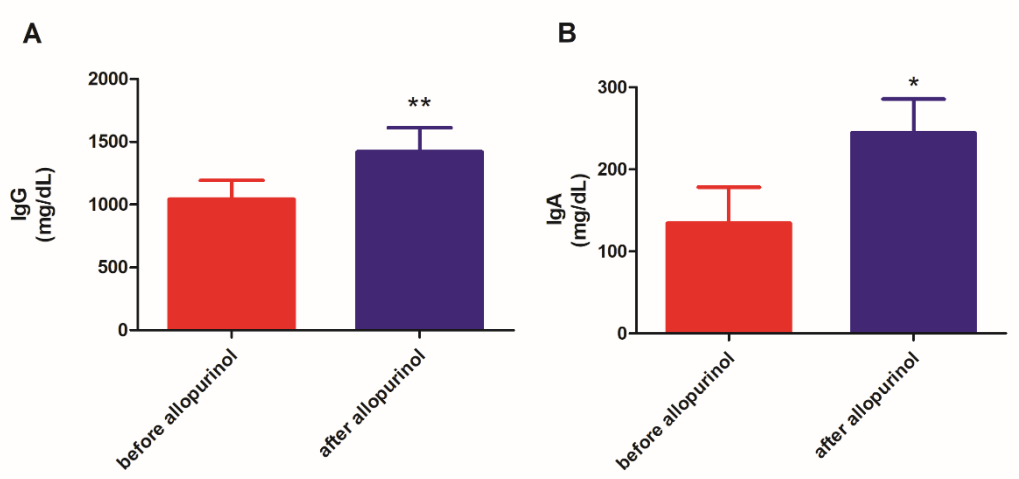

Figure 3 : Effect of XO inhibition on IgG and IgA plasma levels. Data are expressed as mean ± SEM for comparison of IgG (A) and IgA (B) levels before and after treatment with allopurinol for 6 months. * indicates $P < 0.05$, ** indicates $P < 0.01$, Student's one-tailed, paired t-test.

C. Effect of XO Inhibition on Plasma Levels of Comp C3 and Comp C4 in Renal Stone Patients.

Plasma levels of the Comp C3 and Comp C4 are measured in patients with renal stone before and after long inhibition of XO enzyme. Allopurinol treatment has no significant impact on the plasma levels of Comp C3 and Comp C4 (Fig 4 A,B).

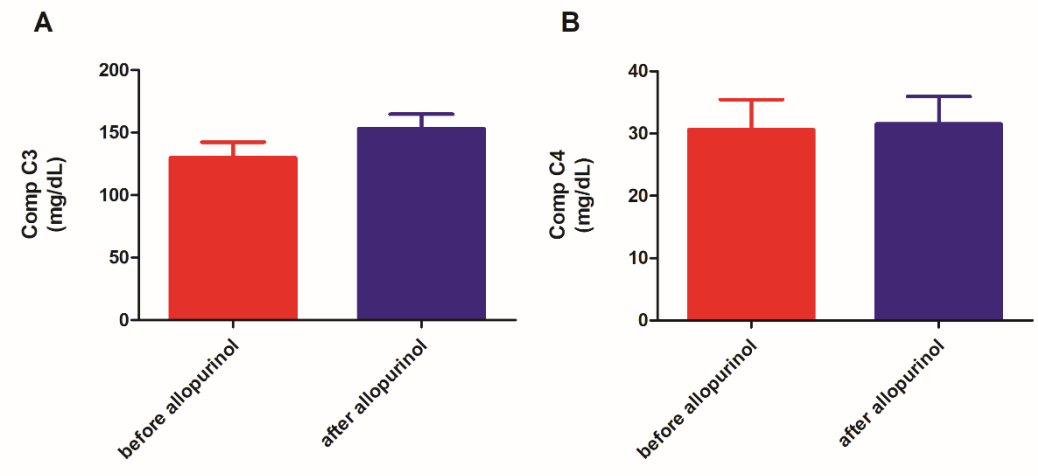

Figure 4: Effect of XO inhibition on Comp C3 and Comp C4 plasma levels. Data are expressed as mean ± SEM for comparison of Comp C3 (A) and Comp C4 (B) levels before and after treatment with allopurinol for 6 months.

4. DISCUSSION:

The present cohort study has demonstrated the impact of xanthine oxidase inhibition on the immunity modulation. Some immunity-related parameters are significantly affected by XO inhibitor (allopurinol) while others are not.

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Inhibition of XO using allopurinol caused a significant inhibition of UA level in the blood. This result is confirmed in human and different animal species [19], [20] and discussed thoroughly in previous reviews [21], [22]. Since metabolism of purines to UA is facilitated by XO, so inhibition of XO is an important way to alleviate hyperuricaemia. Additionally, hyperuricemia can be treated by increasing renal elimination of UA [23], [24]. As xanthine oxidase pathway is one of the most important pathways responsible for generating reactive oxygen species in the body, so XO inhibitors (e.g., allopurinol) can reduce oxidative stress burden and enhance several beneficial biological functions [25]. In contrast, normal level of ROS has positive impact on human health, particularly in neonates. ROS play significant physiological roles by modifying the cell-signalling proteins, and have functional consequences, including cellular proliferation, differentiation and gene expression. [17].

IgA is the most abundant antibody isotype in the body and considered as a less potent opsonin and a weak activator of complement [26]. IgA is a neutralizing antibody that affects the immune responses via protecting mucus epithelium from infectious agents in different parts of the body such as the gastrointestinal tract, the respiratory tract, and the vaginal tract [27]. In contrast, IgG is found in blood and in extracellular fluid, where they have the ability to neutralize and opsonize pathogens for phagocytosis. In addition, IgG is effective in activating the complement system and protecting the extracellular spaces of the internal tissues [26]. Uric acid is regarded as an endogenous adjuvant signal that drives the immune system [28]. A connection between UA and the immunoglobulins was discussed in a recent study by Kanevets et al. (2019) which showed that uric acid precipitation and associated inflammatory response could by provoked by IgM antibody. The authors suggested that this IgM action in increasing the basal level of inflammation can be a novel mechanism of the pathogenesis of gouty arthritis, and UA-enhanced immune activation [29]. However, the relationship between other immunoglobulins and the level of UA are not investigated. The present study showed that the reduction of UA level via using XO inhibitor for 24 weeks caused a significant increase in the level of both IgG and IgA. A study by Kato et al., (2000) which investigated the effect of administration of allopurinol for 14 weeks on immunity in mice showed that after a transient drop in immunoglobulins titre at 2^{nd} week a gradual and steady increase occurred in the level of immunoglobulins till the end of the study (14^{th} week) in which normal levels reached [30]. In addition to the species difference between Kato et al., (2000) study and the present study, our result could be explained by the extended duration of administration of XO inhibitor which made the IgA and IgG levels in the allopurinol-treated group even higher than control.

Reactive oxygen species have bimodal effects on innate immune responses via exerting both beneficial and detrimental actions. ROS play an important role in immune signalling cascade and regulating the redox state of the cells. In contrast, excess of ROS, due to increased production or diminished actions of endogenous antioxidants, can negatively affect the immunity by stimulating extreme biological damage via modifying DNA, proteins, and lipids [31]. Several studies showed that exposure of macrophages to calcium oxalate monohydrate crystals can increase secretion of ROS, chemokines, proinflammatory cytokines, and several fibrotic factors, which facilitates renal interstitial inflammation in kidney stone disease [32]–[34]. Mammalian XO pathway is regarded as an important physiological source of

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superoxide ion, hydrogen peroxide, and nitric oxide, which aid in the activation of various pathways (reviewed in [3]). The present study on human is in line with other studies on different animal models [35], [36] in that inhibition of XO pathway and its related ROS can modulate immunity. A recent study by Kusano et al., (2019) highlighted the physiological importance of XO enzyme in the immune response. The authors reported that in XO knock-in mice a significant increase in tumor growth was recorded compared with wild-type or xanthine dehydrogenase knock-in mice, and that ROS production by XO knock-in macrophages was the mediator of this effect [37]. To be utilized efficiently in host physiology, ROS level should be modulated in both a spatial and temporal manner.

The complement system is primarily associated with the innate immune system which plays a critical role in the modulation of inflammation. Among the complement components, C3 and C4 are particularly important as they are the major plasma proteins of the immune system complement pathways [38]. C3 and C4 can be affected in different immune disorders especially immune complex and some blood associated infectious diseases. Several researches reported that immune disorders that affect the kidney can lead to a reduced level of complements [39]. In infectious disease (e.g., septicaemia and endocarditis) only C3 is often reduced [40]. Whilst both C3 and C4 are often diminished in immune complex disease [41], C4 alone is characteristically decreased in angioedema and vasculitis [42], [43]. In addition, consumption of these complements is considered as a useful prognostic tool which aids in monitoring of some immunological disorders such as nephritis in lupus [44]. However, the present study showed that patients with renal stone had normal levels of both C3 (129.9 ± 12.55) and C4 (30.63 ± 4.810) (normal values for both C3 and C4 are defined as 90-180mg/dl and 20-50mg/dl, respectively). Additionally, the XO inhibition had no significant effect on the circulating C3 and C4 levels.

5. CONCLUSIONS:

The present study highlighted the significant impact of the long-term inhibition of xanthine oxidase on the immunity status. Inhibition of XO in patients with renal stone for 6 months caused increase of the levels of both IgA and IgG, while complements C3 and C4 have not been significantly affected. As only some of the immunity-related parameters are affected by allopurinol, further study is required to investigate other parameters to get a comprehensive view on the impact of XO inhibition on immunity. As the increasing prevalence of hyperuricemia has been recognized as an emerging public health concern, so understanding the role of the XO pathway in modulation of immunity, and the implications of this relationship in chronic renal diseases are important and may facilitate developing better therapeutic strategies in patients with renal diseases.

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Conflict of interest:

None declared.

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