

In Search of a New Screening Test for Osteoarthritis - Is Urinary Nitrate the Answer?

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Abstract

Introduction: Even as early as 1916 it was suggested that the nitric oxide is synthesized in the mammals. This nitric oxide, synthesized in the body, was responsible for vasodilation by the endothelium derived relaxing factors and also by the stimulation of the guanylyl cyclase. Inflammatory conditions increase the production of nitrates and nitrites which is seen in the response to endotoxin and is accompanied with nitrosamine formation. Nitric oxide, hence, has been thought to be the physiological messenger and is increases in the pathological processes. This present study was conducted to find the association between the urinary secretion of nitrates and the osteoarthritis.

Design: A total of 200 patients coming to the hospital out of 100 were patients with osteoarthritis of the knee joint and 100 were randomly selected. All the patients were screened for the causes of secondary osteoarthritis. Urine sample from all the patients was obtained along with X-ray of bilateral knees was taken to assess the grade of osteoarthritis. The osteoarthritic patients were assigned Group A and the randomly chosen patients were assigned the Group B. Both groups were compared and the results were evaluated.

Results: In group A there were 5 cases with urinary nitrate positive as compared to 4 cases with positive urinary nitrate in group B, which was not statistically significant ($p > 0.05$).

Conclusion: The current study clearly shows that there is no association between the urinary nitrate levels and OA.

Keywords: KL grading; Urinary nitrates; Osteoarthritis

Introduction

Even as early as 1916 it was suggested that the nitric oxide is synthesized in the mammals [1]. This was confirmed in many researches done several decades later and it was found that nitric oxide was the excretory product found in mammals [2]. On the determination of the pathogenesis it was found that the root cause for the nitric oxide synthesis was cell toxicity caused with L-arginine [3,4]. This nitric oxide, synthesized in the body, was responsible for vasodilation by the endothelium derived relaxing factors and also by the stimulation of guanylyl cyclase [5,6]. Inflammatory conditions increase the production of nitrates and nitrites which is seen in the response to endotoxin and is accompanied with the nitrosamine formation [7,8]. Nitric oxide, hence, has been thought to be the physiological messenger and is increases in the pathological processes.

There is an increase in the nitrous oxide production seen in animals in conditions like arthritis and nephrotic syndrome [9]. Whereas, there has been an increase in the nitric oxide synthesis in cases of rheumatoid arthritis in humans [10]. Rheumatoid arthritis constitutes the chronic inflammation of the synovium. This chronic inflammation leads to cartilage and bony destruction. This destruction is mediated by metalloproteinases and other superoxide which leads to the production of nitric oxide with the help of nitric oxide synthase [11]. Other inflammatory mediators acting alongside include cytokines, IL-1, TNF and so on also contribute in the production of nitric oxide synthesis. The nitric oxide synthase is of 3 types: endothelial nitric oxide synthase, neuronal nitric oxide synthase and inducible nitric oxide synthase [12,13]. The iNOS is the substance producing the NO in inflammatory conditions like arthritis of the joints [14].

There is very little known about the role and the mechanism of action of the NO in case of arthritis. It is very difficult to assess the NO levels *in vivo* as it is rapidly oxidized into nitrates and nitrites and excreted in the urine [15]. There is not much effect of dietary intake of

nitrates on the urinary nitrates secretion as around 40-50% of nitrates taken in the diet are excreted out in the urine [16,17]. Hence, in the absence of excess of dietary nitrates the urinary nitrates are affected by the inflammatory conditions of the joints [16]. Although many studies have been done to assess the nitrates in inflammatory conditions but studies have only shown the presence of intraarticular nitrates and no study has reported the presence of urinary nitrates in osteoarthritis. This present study was conducted to find the association between the urinary secretion of nitrates and the osteoarthritis.

Material and Methods

A total of 200 patients coming to the hospital out of 100 were patients with osteoarthritis of the knee joint and 100 were randomly selected. A written informed consent was obtained from all the patients. Urine sample from all the patients was obtained along with X-ray of bilateral knees was taken to assess the grade of osteoarthritis using the Kellegren and Lawrence grading (KL grading) [18,19]. The osteoarthritic patients were assigned Group A and the randomly chosen patients were assigned the Group B. A general blood picture along with blood investigations like ESR, CRP, RA factor and S. uric acid were done to rule out any secondary causes of osteoarthritis. If any patients were found to be positive for the above tests, they were removed from the study and another randomly chosen patient was included. A routine

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urine examination was also done to look for any urinary cast or any signs of infection if present. Both groups were compared and the results were evaluated. The calculations were done using the SPSS software version 20 and the paired t test was used to analyse the data obtained.

Results

In group A mean age was found to be 61.32 ± 7.21 years. Whereas, in group B the mean age was 49.76 ± 6.55 years (Figure 1). In group A majority of patients were females ($n = 69$) and in group B the majority of patients were females ($n = 59$) (Figure 2). On assessing the grade of osteoarthritis in group A maximum no. of cases were of grade 3 osteoarthritis ($n = 47$) (Figure 3). In group B maximum no. of cases were of grade 1 of osteoarthritis ($n = 63$) (Figure 3). In group A there were 5 cases with urinary nitrate positive as compared to 4 cases with positive urinary nitrate in group B, which was not statistically significant ($p > 0.05$).

Discussion

In this study the cases (Group A) were of the age group of 61.23 ± 7.21 as compared to the control of the age group of 49.76 ± 6.55 years. The cases were of the age group which was significantly higher as compared to the control group as group A consisted of patients with osteoarthritis which is a degenerative condition seen more commonly in the elderly age group. The majority of patients in both the groups were females as the major inflow of the patients consists of female patients. In group A there were 5 cases who had urinary nitrate positive as compared to 4 cases in group B. Out of the 5 cases in group A with positive urinary nitrate urinary casts were seen in 4 cases whereas 1 case also showed a positive urinary nitrate. In group B all 4 cases showed the presence

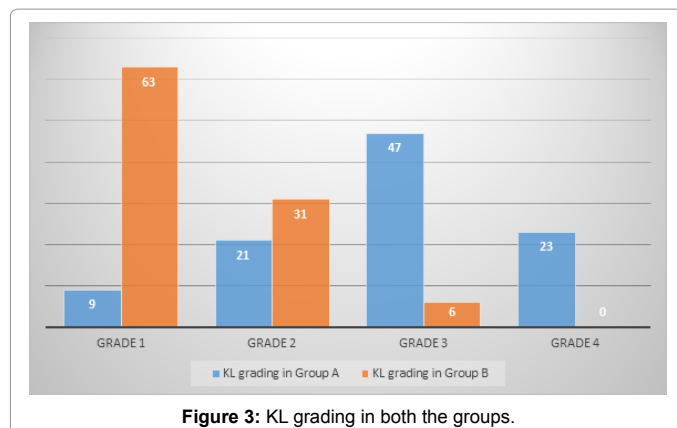


Figure 3: KL grading in both the groups.

of urinary casts. These results were not significant and the presence of urinary nitrate can be attributed to the presence of urinary casts.

The idea behind this study came from previous studies in the literature which clearly indicated the presence of increased nitrates levels in inflammatory arthritis [10]. The mechanism of increased nitrates was given by the production of NO in the synovium membrane in the presence of inflammation. The thought of searching for the nitrate levels in the urine in osteoarthritis was brought upon by the thought that the increased production of NO in the joint may in fact be due to the presence of degeneration in the joint. Animal studies [20] have proven the presence of synovial NO synthesis in cases of osteoarthritis but in our extensive search of the literature there were no cases showing the increase in the urinary nitrate levels in osteoarthritis.

Dirk et al. [10] in 1995 in a study of 10 cases of rheumatoid arthritis showed the presence of increased levels of synovial production of NO which decreases on the administration of steroids. By this they clearly indicated that the NO synthesis in the synovium was part of the inflammatory process and that it could be used as a predictor for the progression of the disease due to the same reason. In the current study it was made sure that all cases of secondary osteoarthritis were ruled out with the help of blood investigations to rule out the inflammatory causes of the increase in the NO synthesis.

On similar lines Gonzalez-crespo et al. [21] assessed the increase in the NO levels in cases of systemic lupus erythematosus. They found the presence of NO in the synovium of patients of SLE which indicated that the NO synthesis was seen in cases with inflammatory arthritis.

The synovial nitrates are only increased in inflammatory conditions and is not increased in trauma as demonstrated by Killian et al. [22] in rabbits with meniscal injury of the knee. The synovial nitrates were evaluated and compared with the levels seen in the normal knee and it was seen that it was no significantly different.

In animal studies done by Spreng et al. [20] it was seen that there was an increase in the NO synthesis in cases of inflammatory as well as osteoarthritis. The inflammatory component was corroborated by the previous studies whereas the increase in osteoarthritis was the cause of further investigation and studies to find its efficacy in human patients.

In a similar study by McInnes et al. [23] it was seen that there was increase in production of synovial nitrates in both rheumatoid and osteoarthritis. But it was not a significant study as the sample size consisted of 7 cases of osteoarthritis and 13 cases of rheumatoid arthritis. All the cases deemed to be osteoarthritis were not assessed for the secondary causes of osteoarthritis and hence, all the cases cannot be deemed to be cases of primary osteoarthritis. The reason for the

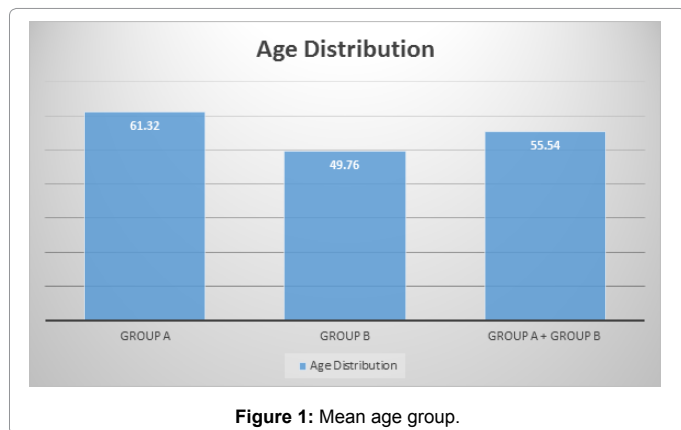


Figure 1: Mean age group.

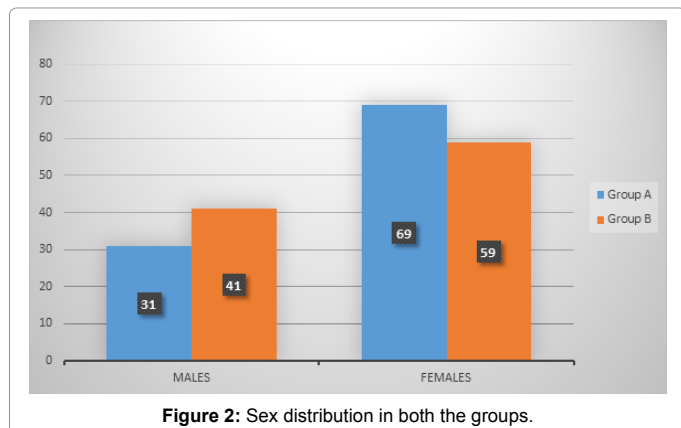


Figure 2: Sex distribution in both the groups.

presence of synovial NO in cases of osteoarthritis was thought to be the presence of inflammation in such cases and no mention of the urinary nitrates has been given in the study. In the current study the synovium has not been assessed and the urinary nitrates have been taken as an indirect marker for the presence of NO in the synovium. Also the large sample size gives an accurate picture that there is no association between osteoarthritis and urinary nitrates and that it cannot be taken as an indirect marker for the diagnosis of osteoarthritis.

In certain recent studies it has been shown that the serum and the synovial nitrates have increased in the cases of osteoarthritis of knee joint. This was seen in a study done by Suantawee et al. [24] where they concluded by saying that the inducible nitric oxide was higher in cases with osteoarthritis as compared to normal individuals. It was also said that this was due to the fact that the nitrogen containing species were involved with the destruction and degeneration of the joint hence, it acted as a good diagnostic marker for osteoarthritis of the knee.

In another study proving indirectly the role of nitrates in osteoarthritis it was seen in a study done by Balaganur et al. [25] that S-methylisothiourea an iNOS inhibitor was seen to be chondroprotective towards the degeneration seen in osteoarthritis. This is very significant and proves the role of the nitrates in osteoarthritis in human population.

These studies indicate the effectiveness of the serum and synovial nitrate levels in the diagnosis and the progression of OA but in the current study it has been seen that there is no correlation between the urinary nitrate levels and diagnosis of osteoarthritis.

A control group was taken from the same area as the study just to rule out any effect of dietary nitrates in the outcome of the study. Hence, the confounding factor of the dietary nitrates being excreted out in the urine was removed. The only fallacy in the current study is that there is no comment on the direct evaluation of the synovial NO levels and its significance in cases of osteoarthritis as with such a large sample size patients and the controls were not willing to undergo any invasive procedure.

Conclusion

The current study along with the studies in the literature clearly show that even though there is a significant association between the serum and synovial nitrate levels but there is no association between the urinary nitrate levels and OA. Hence, the purpose of this study to prove the urinary nitrate levels as an important screening test for OA has failed but with the studies seen in the search of literature we conclude by saying that the serum and synovial nitrates hold promise not only in the future diagnosis of OA but also in assisting in the searching of new avenues in the treatment of a new slowly growing global epidemic i.e. Osteoarthritis.

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Conflict of interests

There is no conflict of interest.

Contributions

Both the authors contributed equally in the conception, data collection, drafting, revision and the finalization of the draft.

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