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SYNTHESIS OF MONOSODIUM URATE CRYSTAL (MSU) FOR GOUT WITH ITS DIAGNOSIS AND CHARACTERIZATION

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ABSTRACT:

Gout is a form of arthritis caused by the accumulation of uric acid crystals in the joints and surrounding tissues. These crystals trigger an inflammatory response, leading to pain and swelling. The exact mechanisms that lead to crystal formation and growth are not fully understood, but researchers have studied the causes of high uric acid levels and the inflammatory pathways involved. Uric acid, the end product of purine metabolism, is normally present as the urate anion in the body fluids. It combines with sodium ions to form MSU crystals, which have a unique triclinic structure and appear needle-shaped under a microscope. Diagnosis of gout is crucial and involves various methods such as polarizing light microscopy, dual-energy computed tomography (DECT), ultrasonography (US), and urine analysis. Treatment options include nonsteroidal anti-inflammatory drugs (NSAIDs) to reduce inflammation, as well as xanthine oxidase inhibitors and uricosuric substances to lower uric acid levels and prevent crystal formation. Researchers have also developed techniques for synthesizing MSU crystals in vitro, such as the single diffusion gel technique and wet chemical precipitation technique. These crystals can then be characterized using techniques such as Fourier-transform infrared spectroscopy (FTIR), powder X-ray diffraction (XRD), and thermogravimetric analysis (TGA). By understanding the epidemiology, disease mechanisms, diagnosis, treatment, and in vitro synthesis of MSU crystals, researchers aim to improve the management and prevention of gout.

Keyword: Gout, Arthritis, Inflammation, FTIR, XRD, TGA, MSU, etc.

INTRODUCTION:

Gout, a type of arthritis, results from monosodium urate (MSU) crystal deposits within joints and tissues, causing inflammation. While high levels of serum urate and resulting inflammatory pathways are understood, the mechanisms leading to crystal formation and growth remain unclear. Uric acid, a by-product of purine metabolism, is normally present as a negatively charged urate ion, combining with sodium to form MSU. In gout, MSU crystal accumulation triggers intense inflammation. Crystallization occurs when urate levels in the blood exceed the saturation point, increasing the risk of crystal formation. This can stem from an imbalance between urate production and excretion or a combination of both. Overproduction of uric acid can arise from increased purine biosynthesis or excessive cellular turnover. Chronic hyperuricemia (high urate levels) can lead to kidney problems, kidney stones, and gouty arthritis in susceptible individuals.

1. GOUT AND MONOSODIUM URATE CRYSTALS:

Uric acid is a weak organic acid with two pKa values, 5.75 and 10.3. Under normal physiological conditions, uric acid exists in the blood and joint fluid as a singly deprotonated ion. When uric acid crystallizes, it can take various forms, including fully protonated uric acid (found in kidney stones) and salts created by deprotonated or partially deprotonated uric acid. One common crystallized form is monosodium urate monohydrate (NaC5H3N4O3·H2O), which is formed when a urate molecule binds with sodium and water molecules. Monosodium urate monohydrate is the main deposit observed in gouty arthritis, although uric acid can also precipitate with other mineral phases.[1]

2. OVERVIEW OF GOUT:

Gout is a condition caused by the buildup of uric acid crystals in the joints, tendons, and skin. This buildup is due to high levels of uric acid in the blood, which can be caused by a number of factors, including diet, genetics, and certain medical conditions. Gout can cause a variety of symptoms, including pain, swelling, redness, and stiffness in the joints. In severe cases, gout can also damage the joints and lead to disability.[2]

2.1. Epidemiology of Gout:

Gout, the most common type of inflammatory arthritis, has shown an increase in prevalence and incidence in recent years, as indicated by multiple studies. Various risk factors for gout development in both men and women have been identified through epidemiological research, including hyperuricemia, genetic and dietary factors, alcohol consumption, metabolic syndrome (involving hypertension and obesity), diuretic usage, and kidney disease. While osteoarthritis (OA) may lead to local deposition of MSU crystals, it is not a direct risk factor for gout development. Gout has been found to independently contribute to overall and cardiovascular mortality and morbidity, surpassing the impact of traditional cardiovascular risk factors associated with the condition.[3]

A) Signs and symptoms of Gout:

Severe joint pain, Persistent unease, Swelling and irritation, Restricted movement of joints.

B) Causes:

Factors that can contribute to an increased risk of developing gout include elevated urate levels, a family history of the condition, alcohol consumption, a diet high in purine-rich foods, intake of beverages containing high-fructose corn syrup, being overweight or obese, having high blood pressure, and suffering from chronic kidney disease.

3. MSU CRYSTALS:

Monosodium urate (MSU) is a common salt of uric acid. Uric acid is a byproduct of purine metabolism and is normally excreted through the kidneys or gastrointestinal tract. MSU crystals can form in the renal tubules and ureters, leading to kidney stones, or in the synovial tissue or soft tissues. The exact cause of MSU crystal formation in the joints is unknown. Acute gout attacks are caused by the inflammatory response to MSU crystals. Neutrophils are activated by MSU crystals and release inflammatory mediators, leading to the inflammation associated with gout.[4]

3.1. Synthesis of Monosodium Urate (MSU) Crystals:

Monosodium Urate Monohydrate (MSU) crystals were prepared by following a method:

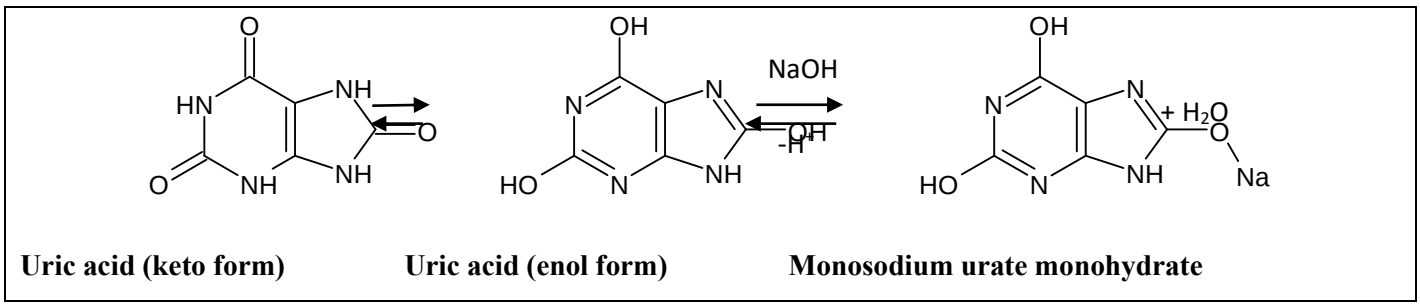
1) Uric acid (8 g) was dissolved in 1,600 ml of distilled water with 49 ml of NaOH (1N) at 60°C continuously stirred.

2) The moderately alkaline solution's pH was neutralized to 7.2 by adding 1N HCL.

3) To produce superior crystals, the solution was cooled with gentle swirling at ambient temperature. It was subsequently refrigerated at 5°C overnight or set aside at room temperature for leisurely crystal development.

4) The crystals were cleansed by carefully removing the overlying liquid and subsequently rinsed and dried.

5) The crystal form was confirmed through microscopic observation.



Uric acid (keto form) **Uric acid (enol form)** **Monosodium urate monohydrate**

Fig. No. 1 Conversion of uric acid to Monosodium urate

3.2.Microscopic Observation of MSU Crystal:

The microscopic observation of prepared Monosodium urate crystals was observed under light microscope the result obtained are given below,

Fig. No. 2 Microscopic Observation of MSU Crystal

Monosodium urate, a salt formed by uric acid ions, consists of one sodium ion, one water molecule, and one urate molecule bonded together. Under polarized light, MSU crystals exhibit strong negative birefringence and appear as needle-shaped crystals with lengths ranging from 5 to 25 micrometers. These crystals exhibit a triclinic crystal structure, featuring faces along three unequal axes that are not perpendicular to each other.

4. ROLE OF MSU CRYSTAL IN PATHOGENESIS OF GOUT

Gout develops and manifests in three stages, which are as follows:

(a) Chronic Hyperuricemia, (b) The growth of Monosodium Urate (MSU) Crystals, and (c) Interaction between MSU Crystals and The Inflammatory System.[5]

4.1. Pathogenesis of Hyperuricemia:

Gout develops through four phases:

1. Asymptomatic Hyperuricemia: High uric acid levels in the blood, but no symptoms or crystal deposits.

2. Crystal Formation: Uric acid crystals form in the body, but without causing gout symptoms.

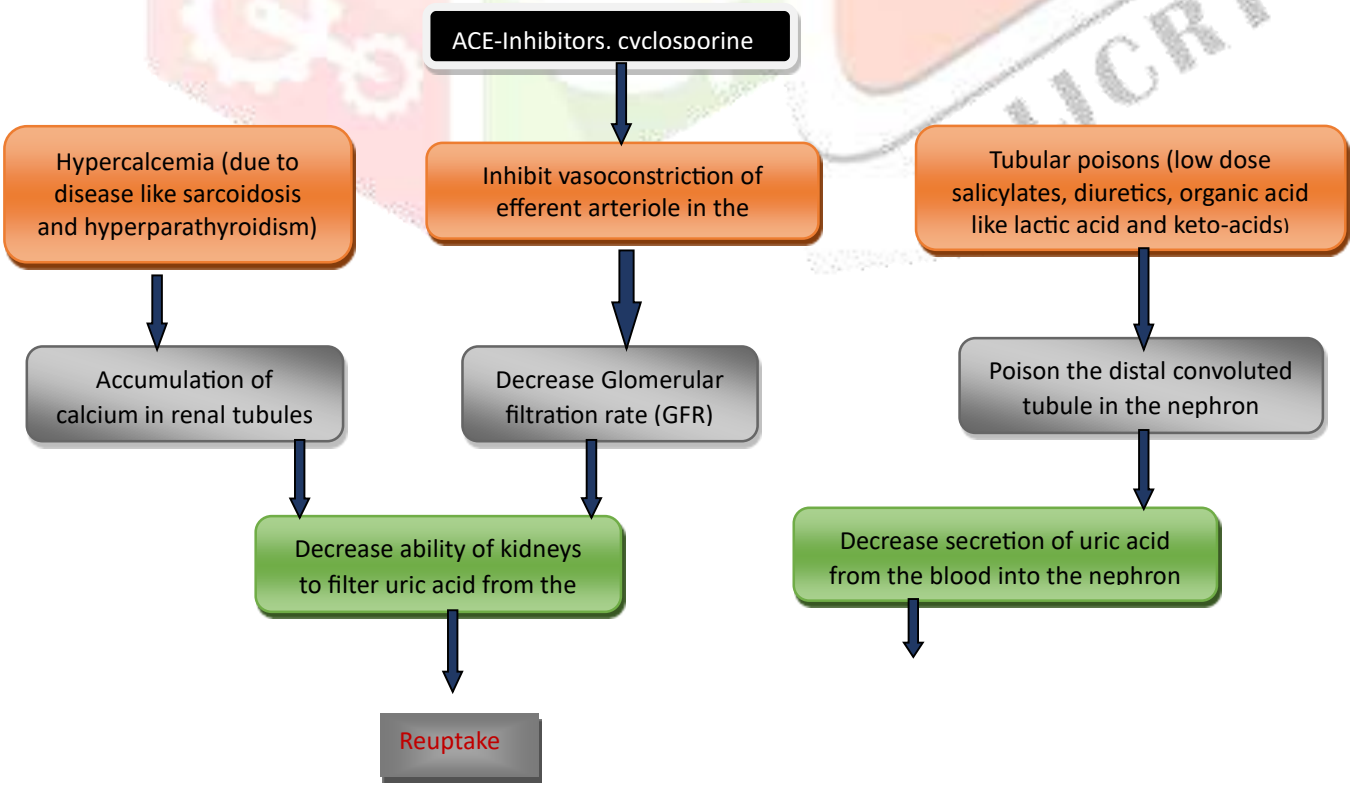
3. Intermittent Gout: Crystals accumulate, leading to occasional gout attacks.

4. Advanced Gout: Characterized by tophi (crystal deposits beneath the skin), persistent joint swelling, and bone damage.

Hyperuricemia:

Hyperuricemia refers to elevated uric acid levels in the bloodstream. Pathological hyperuricemia occurs when uric acid levels exceed the threshold for crystal formation (6.8 mg/dL). This can result from increased uric acid production, reduced excretion (due to factors like kidney disease, medications, or diet), or both. Rapid cell breakdown (e.g., from blood cell destruction or tumor dissolution) can also raise uric acid levels. In gout, impaired uric acid excretion is the primary cause of hyperuricemia. Approximately two-thirds of uric acid elimination occurs via the kidneys, while the remaining one-third is eliminated through the gastrointestinal tract.

Active tubular secretion and reabsorption processes occur throughout the proximal renal tubule, with about 10% of initially filtered uric acid ultimately excreted. This process is regulated by a set of apical and basolateral secretory and reabsorptive proteins, some of which are targets for drugs that lower uric acid levels. These proteins can be classified into reabsorptive urate-anion exchangers (like URAT1/SLC22A12, OAT4/SLC22A11, and OAT10/SLC22A3), the reabsorptive GLUT9/SLC2A9 urate transporter, secretory anion exchange transporters (like OAT1, OAT2, and OAT3), sodium phosphate transporter proteins (including NPT1/SLC17A1 and NPT4/SLC17A3), and the ATP-driven secretory efflux pump MRP4/ABCC4. Within the intestinal tract, the secretory transporter ABCG2 plays a significant role. Reduced functionality of ABCG2 can lead to diminished extra-renal excretion, resulting in a compensatory increase in urinary uric acid output.



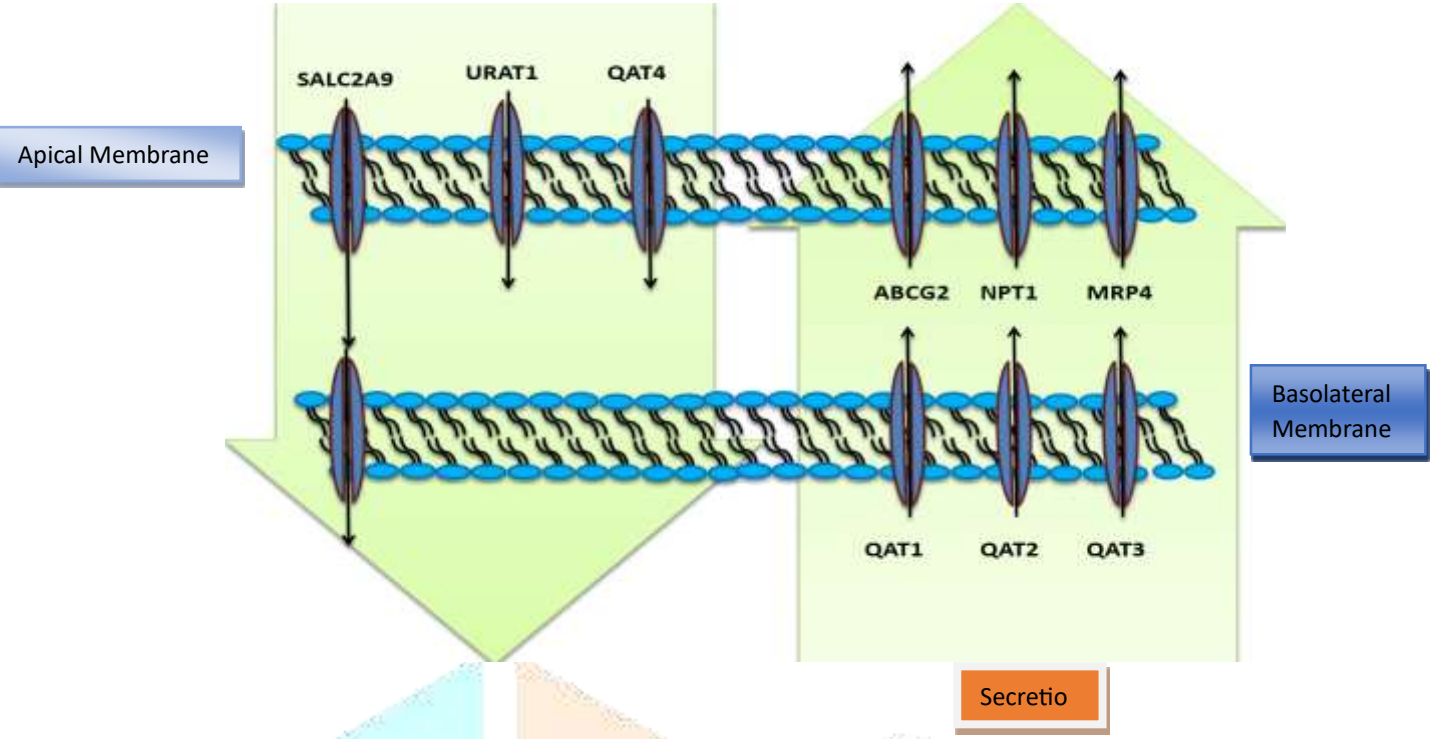


Fig. No. 3 Hyperuricemia caused due to renal underexcretion.

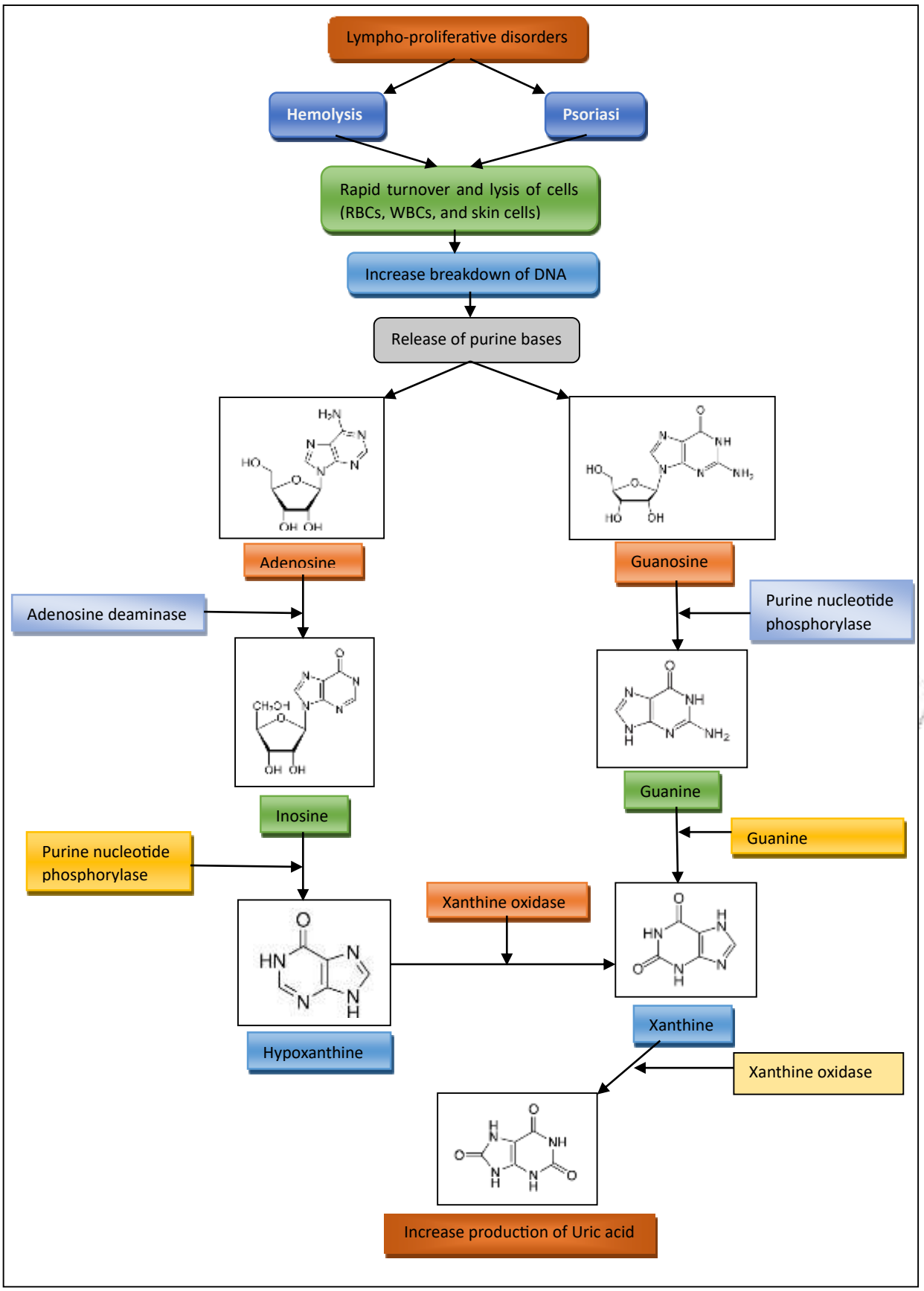


Fig. No. 4 Hyperuricemia caused due to increased hepatic metabolism.

5. DIAGNOSIS OF MSU CRYSTALS PRESENCE IN GOUT

5.1.Polarising Light Microscopy:

The best way to diagnose gout is to see needle-shaped monosodium urate crystals under a special microscope called a polarizing microscope. These crystals are 1-20 micrometers long and will appear negatively birefringent, meaning they will appear to have different colors when rotated under the microscope. During an acute gout attack, the joint fluid will appear yellow, cloudy, and not thick, with a large number of white blood cells (sometimes more than 50,000 cells per cubic millimeter), mostly neutrophils. Although monosodium urate crystals are most often found after aspirating fluid from a joint that is acutely inflamed during a flare, these crystals are also often found in the joints of patients with gout who do not have any symptoms, especially in joints that have been inflamed before. [6]

5.2.Ultrasound:

An ultrasound diagnostic crystal (DCS) or hydroxyapatite (HCA) was seen in 11 of 26 patients, while monosodium urate (MSU) crystals were identified in 9 patients. On ultrasound, sensitivity was 100%, specificity was 88.2%, positive predictive value was 81.8%, negative predictive value was 100%, and the positive likelihood ratio was 5.5. Agreement between radiologists reading the ultrasound images was fair to very good, with a kappa coefficient of 0.79–0.93. [7]

5.3.Dual-Energy Computed Tomography (DECT)

In the early stages, the US technique was significantly more effective than DECT in detecting MSU deposits (66.7% vs 26.6%, $p \geq 0.05$). However, in the middle and late stages, both US and DECT methods exhibited similar sensitivities. For the early-stage group, the US examination yielded positive results for nine joints: double contour sign in four, snowstorm sign in four, and both double contour sign and snowstorm sign in one. Conversely, DECT did not identify any urate crystal deposits in this early-stage group.[8]

6. TREATMENT OF GOUT

Gout, the most prevalent rheumatic disease, is readily managed. Maintaining low uric acid levels throughout one's lifetime can dissolve problematic crystals and alleviate associated symptoms.

- Xanthine oxidase inhibitors reduce uric acid in the blood by blocking its production and improving its elimination, no matter how the body processes uric acid. Allopurinol is the most common and oldest xanthine oxidase inhibitor. Allopurinol and its active metabolite, oxypurinol, are purine-like molecules that block the breakdown of purines and pyrimidines by competing with xanthine oxidase for binding and inhibiting it and other enzymes to a lesser extent. Febuxostat is a non-purine, non-competitive drug that also inhibits xanthine oxidase. Unlike allopurinol, febuxostat does not affect other enzymes and prevents the formation of both oxidized and reduced forms of xanthine oxidase. Xanthine oxidase inhibitors, when given in appropriate doses, can be taken over the long term to both prevent urate crystals from forming and dissolve existing ones, including tophi. Surprisingly, the rate of crystal breakdown often goes down as uric acid levels are lowered.

- Uricosuric substances can help prevent the reabsorption of uric acid in the kidneys. This helps to remove uric acid from the body and can help to treat conditions like gout. Probenecid and leisured are two uricosuric medications that have been approved by the FDA for use in the United States.
- In order to dissolve MSU crystals, the level of SUA needs to be lowered to levels that are lower than the saturation point of MSU. The ACR and EULAR recommend that all gout patients aim for a SUA target of less than 6 mg/dL, and less than 5 mg/dL for those with severe gout, to speed up the reduction of crystal buildup.
- Gout flare treatments consist of colchicine, nonsteroidal anti-inflammatory drugs (NSAIDs), and steroids, which can be administered together in severe cases and are most beneficial when administered shortly after the flare begins. Nonsteroidal anti-inflammatory drugs (NSAIDs) are a type of pain reliever that can alleviate inflammation and pain associated with gout attacks.
- In certain situations where non-steroidal anti-inflammatory drugs (NSAIDs), colchicine, or steroids cannot be used or are ineffective, medications known as interleukin-1 (IL-1) receptor antagonists come into play. Canakinumab, a long-lasting antibody against IL-1 beta, has been given the go-ahead for use by the European Medicines Agency. [9]

7. IN-VITRO SYNTHESIS OF A MONOSODIUM URATE (MSU) CRYSTALS

❖ **Single Diffusion Gel Growth Technique:**

(a) Gel preparation:

As earlier mentioned in the section 5.3 of Chapter-V, the concentrated solution of sodium meta-silicate was prepared. By adding double distilled water of appropriate volume into the concentrated solution, the solution of the desired specific gravity was prepared. In the present study, the specific gravity of sodium meta-silicate solution was selected as 1.05 and equal volume of 0.2 M, NaOH solution was added to prepare a mixture. This mixture was acidified by 2 N, acetic acid in such a manner that the appropriate pH was obtained. This mixture was then used to set the gel. In the present study, the pH values were selected within 4.5 to 5.0.

b) Wet Chemical Process:

The MSUM crystals were grown using the wet chemical precipitation method. In this procedure, a solution with a concentration of 0.2 M NaOH was agitated and heated to 70 degrees Celsius. Once the desired temperature was reached, uric acid was introduced, creating a suspension within the NaOH solution. Initially, the uric acid dissolved due to its weak acidic nature and the highly basic NaOH solution. However, after adding a sufficient amount, the solution transformed into a suspended uric acid solution. The mixture was stirred continuously at a constant temperature for four hours, resulting in the formation of fine MSUM crystal particles at the bottom. Although the coagulated particles appeared amorphous and porous, the crystalline nature was confirmed through powder XRD analysis. The likely reaction responsible for the MSUM formation is outlined as follows.[7]

NaOH + C5H4N4O3 ¬ NaHC 5H2N4O3· H2O

8. IN VIVO STUDY OF MSU CRYSTAL-INDUCED INFLAMMATION

Injection of 10 milligrams of MSU crystals into the pouch resulted in an immediate exudate. The exudate volume and white blood cell count peaked at 24 hours and gradually decreased over the following 3 days, mirroring the natural course of acute gout. Membrane thickness exhibited a similar trend. The linear densities of polymorphonuclear cells (PMN) in the membrane were closely associated with exudate WBC counts, indicating PMN recruitment from the subintimal synovial membrane. Within 2 hours of crystal injection, both monocyte/macrophage and mast cell linear densities increased in the subintimal layer ($P < 0.038$ and $P < 0.03$, respectively, compared to controls), while PMN linear densities displayed 2 peaks at 4 hours and 24 hours. The exudate's histamine content reached its peak 6 hours post-injection, coinciding with minimal mast cell linear densities in the membranes, suggesting mast cell degranulation.[8]

9. SYNTHESIS OF MONOSODIUM URATE (MSU) NANO PARTICLES AND ITS CHARACTERIZATION

Gout is a condition characterized by the build-up of Monosodium Urate (MSU) crystals in various connective tissues and joints, leading to considerable pain and inflammation. The initial creation of nano particles is believed to play a role in the formation of micro-particles, underscoring the significance of producing and characterizing MSU nano particles. These nano particles were generated using a wet chemical technique involving NaOH and uric acid (C5H4N4O3), and subsequently examined through powder XRD, TEM, FT-IR, and thermal analysis methods. Analysis through powder XRD unveiled a triclinic structure, with an average particle size of 40 nm determined through Scherrer's formula. TEM analysis indicated a particle size range of 20 to 60 nm. The FT-IR spectrum of the MSU nano particles verified the existence of several vibrations including O-H stretching, N-H stretching, N-H rocking, C = O, C = C Enol or Keto, and C = N. Thermal analysis conducted from room temperature to 900ºC demonstrated that the thermal stability of MSU nano particles was marginally higher in comparison to bulk MSU, with 1.5 water molecules linked to the MSU nano particles. These results were then juxtaposed with those of bulk MSU to gain further insights. Gout is a condition characterized by the build-up of Monosodium Urate (MSU) crystals in various connective tissues and joints, leading to considerable pain and inflammation. The initial creation of nano particles is believed to play a role in the formation of micro-particles, underscoring the significance of producing and characterizing MSU nano particles. These nano particles were generated using a wet chemical technique involving NaOH and uric acid (C5H4N4O3), and subsequently examined through powder XRD, TEM, FT-IR, and thermal analysis methods. Analysis through powder XRD unveiled a triclinic structure, with an average particle size of 40 nm determined through Scherrer's formula. TEM analysis indicated a particle size range of 20 to 60 nm. The FT-IR spectrum of the MSU nano particles verified the existence of several vibrations including O-H stretching, N-H stretching, N-H rocking, C = O, C = C Enol or Keto, and C = N. Thermal analysis conducted from room temperature to 900ºC demonstrated that the thermal stability of MSU nano particles was marginally higher in comparison to bulk MSU, with 1.5 water molecules linked to the MSU nano particles. These results were then juxtaposed with those of bulk MSU to gain further insights.[10]

10. CHARACTERIZATION OF MONOSODIUM URATE (MSU) CRYSTAL

❖ XRD

The MSU nanoparticles were examined using powder X-ray diffraction (XRD) with Cu Kα radiation on a PHILIPS X'PERT MPD setup. Subsequently, the XRD pattern was evaluated with powder X software, revealing the crystallographic planes of the MSU nanoparticles. The analysis indicated that MSU exhibits triclinic crystal structure, with unit cell dimensions of a = 10.5001 Å, b = 9.5120 Å, and c = 3.412 Å, as well as angles α = 95.06°, β = 99.47°, and γ = 97.14°. The average size of the crystallites was determined to be 40nm using Scherrer's formula.

❖ FTIR

The analysis using FT-IR was conducted on a Shimadzu FT-IR 8400 within the range of 400 cm-1 to 4000 cm-1 using a KBr disc medium. The presence of water molecules is primarily indicated by a distinct decrease at 3599 cm-1, attributed to O-H stretching. The wide peak at 3048 cm-1 signifies N-H stretching, while absorptions between 725 cm-1 to 850 cm-1 indicate N-H rocking. A broad decrease in absorption at 1680 cm-1 suggests the existence of multiple C=O vibrations, with absorptions at 1528 cm-1 corresponding to C = C in enol or keto forms. The absorptions at 1260 cm-1 and 1387 cm-1 relate to C-N vibrations. The presence of oxygen-metal vibrations is observed between 400 cm-1 to 600 cm-1. A comparison is made between the absorptions of bulk MSUM and nano MSU. In bulk, C=O vibrations occur at 1737.7 cm-1, whereas in nano MSU, they appear at 1680 cm-1 with a red shift. The bulk C=C vibration is seen at 1500.5 cm-1, while in nano MSU, it is at 1528 cm-1 with a blue shift. O-H vibrations at 3598 cm-1 and 3599 cm-1 for bulk and nano forms respectively indicate no shift. Previous findings suggest that shifts in the FTIR spectrum are observed due to particle size reduction, leading to red or blue shifts.

❖ TGA:

It is evident that the compound shows stability up to 210ºC, with minimal weight loss of only 4%. Subsequently, dehydration begins at 260 ºC, resulting in a weight loss of 12.4%, followed by another stable phase up to 350 ºC with a weight loss of just 2.9%. At 400 ºC, a significant decomposition reaction occurs, leading to a substantial 61.1% weight loss. A third reaction transpires between 500 ºC and 655 ºC, resulting in a 7.1% weight loss, while the residual compound remains thermally stable at 700 ºC. The MSU nano-particles undergo dehydration initially, followed by decomposition, culminating in a weight loss of 87.5% of the original weight at 700 ºC. In contrast, the crystalline MSUM begins dehydrating at 170 ºC and completes dehydration at 240 ºC, retaining 87.20% of the original weight at 700 ºC. The thermo-gram analysis revealed that 1.507 water molecules are associated with the MSU nano-particle sample, indicating that the water molecules are slightly more tightly bound to the nano-particles, possibly due to the larger surface area and higher surface energy characteristic of nano-particles.

❖ DSC AND DTA:

DSC and DTA plots of MSU nanoparticles displayed two endothermic reactions at temperatures of 251 ºC and 558.6 ºC, as well as three exothermic peaks observed at 427.6 ºC, 473 ºC, and 672.1 ºC. The software TA evaluation provided with the setup was utilized to calculate various thermodynamic parameters from the plots. The initial endothermic reaction is likely attributed to dehydration, with enthalpy and change in heat capacity values of -203.72 J/g and 0.67 J/gK. Similarly, the second endothermic peak at 558.6 ºC may indicate slow decomposition with marginal weight loss, exhibiting enthalpy and change in heat capacity values of -272.84 J/g and 3.60 J/gK. The two exothermic reactions at 427.6 ºC and 473 ºC suggest a phase change, with enthalpy and change in heat capacity values of 138.33 J/g, 0.99 J/gK and 125.11 J/g, 0.66 J/gK, respectively. Another exothermic reaction at 672.1 ºC may result from the complete decomposition of the sample into the gaseous phase with reaction within the products, showing enthalpy and change in heat capacity values of -616.26 J/g and 1.27 J/gK. The heat change values measured were -95.70 µVs/mg and -89.11 µVs/mg for the temperatures of 251 ºC and 558.6 ºC, respectively. The heat change values for the three exothermic reactions occurring at 427.6 ºC, 473 ºC, and 672.1ºC were 51.32 µVs/mg, 44.46 µVs/mg, and 18.50 µVs/mg, respectively.[11]

CONCLUSION:

The formation of MSU crystals is greatly influenced by an environment with high levels of urate. While various factors can decrease the solubility of MSU, aid crystal formation, or accelerate crystal growth, they may contribute to urate crystallization. Understanding the fundamental chemistry and the impact of proteins, particularly immunoglobulins, on the development of MSU crystals could lead to innovative and potentially more effective treatments for gout patients and other crystal-related conditions. Diagnostic methods for MSU crystals include polarising light microscopy, dual-energy computed tomography (DECT), and ultrasonography (US). Treatment options involve nonsteroidal anti-inflammatory drugs (NSAIDs), xanthine oxidase inhibitors to inhibit urate synthesis and lower blood uric acid levels, and uricosuric agents to block renal transporters. In vitro synthesis methods for MSU crystals include the single diffusion gel technique and wet chemical precipitation technique. Characterization techniques such as Fourier transform infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD), and thermogravimetric analysis are used to analyze MSU crystals.

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