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CORRELATION BETWEEN PRE AND POST TREATMENT EFFECT OF METHOTREXATE AND ANTI-OXIDANT STATUS IN RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Rheumatoid Arthritis (RA) is a chronic inflammatory disorder characterized by oxidative stress and joint damage. This study evaluates the biochemical impact of Methotrexate (MTX) treatment on RA patients, focusing on oxidative stress markers, antioxidant activity, and overall disease management. Analysis of 60 RA patients before and after MTX treatment revealed significant biochemical changes. Post-treatment, Malondialdehyde (MDA), a marker of oxidative stress, was elevated, while Glutathione (GSH) levels increased, indicating some restoration of antioxidant defense. However, catalase and superoxide dismutase (SOD) activities were significantly reduced, reflecting persistent oxidative imbalance. Non-enzymatic antioxidants, including vitamins A, C, and E, showed substantial improvement post-treatment. Elevated Advanced Oxidation Protein Products (AOPP) and reduced nitric oxide (NO) levels post-treatment suggest decreased inflammatory activity. Electrolyte levels, specifically sodium and potassium, were notably altered, indicating potential disruptions in homeostasis. Additionally, increased urea and creatinine levels post-treatment point to potential renal dysfunction. This study underscores Methotrexate's role in modulating oxidative stress and inflammation in RA, highlighting the importance of monitoring biochemical parameters for effective disease management and the need for further research into long-term treatment effects and management strategies.

Keywords: Rheumatoid Arthritis, Chronic Inflammatory Disorder, Treatment, Pre-treatment, Post-treatment, Management Strategies, Methotrexate, Anti-oxidant.

INTRODUCTION:

Bone remodelling is a complex process orchestrated by osteoblasts, which form new bone, and osteoclasts, which break down bone. Any imbalance in this cycle due to biological changes such as those influenced by inflammatory cytokines, growth factors, or hormones can lead to skeletal abnormalities like osteoporosis and osteoporosis. [1] Osteoporosis is a prevalent condition, especially in the elderly, women, bedridden patients, and astronauts who experience bone loss due to a lack of gravity. This condition, characterized by reduced bone density, can result in fractures. [2]

Rheumatoid Arthritis (RA) Overview:

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease, primarily affecting the synovium (the lining of joints). In developed regions, RA affects about 0.5% to 1% of the adult population. The disease's progression varies widely, ranging from mild to severe, and is often accompanied by joint damage, disability, and comorbidities. [5] Mortality rates in RA patients are notably higher than in the general population, and the disease's progression can be unpredictable, often presenting with intense pain, fatigue, and reduced quality of life. [6]

The synovial fluid of inflamed rheumatoid joints is rich in inflammatory cells, including neutrophils, which produce reactive oxygen species (ROS) such as hydrogen peroxide (H2O2) and superoxide radicals. These ROS are significant contributors to oxidative damage, affecting lipids, proteins, and DNA within the joint. In RA patients, elevated oxidative stress leads to lipid peroxidation and protein oxidation, worsening the disease. Antioxidants such as glutathione, superoxide dismutase (SOD), and glutathione peroxidase counteract these harmful effects, but their activity is often reduced in RA patients, contributing to disease progression. [7] To protect against oxidative damage, cells utilize various antioxidant systems. Enzymes like SOD convert superoxide radicals into less harmful substances, while glutathione peroxidase helps neutralize hydrogen peroxide. [8] However, in RA, these antioxidant defense are compromised. Studies have shown increased oxidative damage markers in RA patients, with alterations in various oxidant and antioxidant enzyme activities. [9]

RA pathogenesis is driven by the overproduction of inflammatory mediators such as reactive nitrogen species (RNS) and ROS. These species play a crucial role in cartilage degradation and joint destruction. [10] The presence of nitric oxide (NO), produced by nitric oxide synthase, contributes to the oxidative environment in RA joints. The accumulation of ROS and RNS leads to cellular damage, particularly in the cartilage and bone. [11] RA is characterized by the formation of a pannus, a fibrous tissue that invades and destroys cartilage and bone. This leads to bone erosion at the interface between the pannus and the subchondral bone. If left untreated, bone erosion can progress rapidly, leading to joint deformity and loss of function. Preventing bone erosion is a critical therapeutic goal in RA management. [12] [13] Depression is a common comorbidity in RA, affecting 13% to 42% of patients. This mental health condition can be exacerbated by the physical limitations and chronic pain associated with RA. Depression in RA patients often presents with fatigue, sleep disturbances, and a reduced quality of life. Treating depression is essential for improving overall outcomes in RA patients, as untreated depression can worsen the disease. [14] Although the exact cause of RA remains unclear, genetic predisposition plays a significant role. More than 80% of RA patients carry the HLA-DRB1*04 genes, which increases their risk of developing the disease. Additionally, environmental factors such as smoking can accelerate the progression and severity of RA. [15] Other associated genetic loci, such as PTPN22 and STAT4, contribute to disease risk. [16]

Tumor necrosis factor (TNF)-α and Interleukin-1 (IL-1) are key inflammatory cytokines involved in RA pathogenesis. These cytokines promote synovitis (inflammation of the synovium) and stimulate osteoclastic bone resorption, leading to bone erosion. Targeting TNF-α has been one of the most successful strategies in modern RA treatment, with TNF inhibitors like adalimumab, infliximab, and etanercept significantly improving patient outcomes. [17] Recent advances in RA treatment focus on

biologic agents that target specific components of the immune system. These include monoclonal antibodies, fusion proteins, and cytokine inhibitors that disrupt the inflammatory pathways responsible for RA progression. [19] The goal of RA treatment is to control inflammation, prevent joint damage, and maintain functionality while minimizing side effects. Early initiation of disease-modifying antirheumatic drugs (DMARDs) is crucial for preventing long-term damage, with combination therapies often proving more effective than single-drug treatments. RA patients are at a higher risk of cardiovascular diseases, including coronary artery disease (CAD). The inflammatory processes in RA contribute to atherosclerosis and increase the likelihood of heart-related complications. Management of cardiovascular risk factors is an essential component of RA care, as heart disease accounts for a significant portion of the increased mortality in RA patients. [18] [20] The management of RA has evolved significantly over the past decades, with a shift toward early, aggressive treatment to prevent joint damage and improve quality of life. Advances in understanding the genetic, environmental, and immunological factors driving RA have led to the development of targeted therapies that offer new hope for patients. As research continues, the goal remains to achieve remission and halt disease progression while addressing comorbidities such as depression and cardiovascular risk. [21]

Aims and Objectives

1. **Evaluation of Biochemical Response of Methotrexate in Rheumatoid Arthritis (RA) Patients:** This objective aims to assess how Methotrexate, a common disease-modifying anti-rheumatic drug (DMARD), influences the biochemical parameters in patients suffering from RA. The focus is on understanding the drug's role in modulating the biochemical markers associated with the disease and its progression.
2. **Assessment of the Correlation between Methotrexate and Antioxidant Activity:** The study intends to explore the relationship between Methotrexate treatment and the body's antioxidant mechanisms. RA patients often experience oxidative stress, which plays a role in the pathogenesis of the disease. This objective seeks to determine how Methotrexate influences antioxidant levels and activity in the body, potentially mitigating oxidative damage.
3. **Investigation of the Link between Methotrexate and Rheumatoid Arthritis:** This objective is focused on determining the broader therapeutic implications of Methotrexate in managing RA. It aims to explore the overall efficacy of Methotrexate in controlling the symptoms and progression of RA, as well as its role in improving clinical outcomes for patients.

LITERATURE SUPPORT:

Kuhn et al. elucidated that in osteoarthritis (OA) patients, chondrocytes produce higher levels of inflammatory cytokines like IL-1β and TNF-α, which reduce collagen synthesis and increase degradative proteases, including matrix metalloproteinases (MMPs). Additional inflammatory regulators such as IL-6, IL-8, prostaglandin E2, and nitric oxide also contribute to disease progression [22]. Jorge emphasized the rising evidence that estrogens play a role in joint tissue activity via complex molecular pathways, countering previous assumptions that articular tissues are insensitive to estrogens [23]. Free radicals play a significant role in the normal regulatory systems, and their dysregulation is implicated in inflammation [24]. Elena et al. observed that synovial fibroblasts in rheumatoid arthritis (RA) contribute to joint erosion and inflammation. Despite recent insights into fibroblast activation through matrix degradation, inflammatory factors, and epigenetic modifications, the early pathophysiological processes leading to chronic RA remain unclear. Sermin further explained that reactive oxygen and nitrogen species can directly damage DNA, hindering repair mechanisms [25] Davis et al. found that osteoarthritis (OA) patients exhibit increased expression of Wnt pathway genes, suggesting a role in subchondral bone changes. Velasco noted significant differences in genetic predisposition to RA and emphasized the importance of early disease-modifying antirheumatic drugs (DMARDs) treatment. Arnett et al. established the diagnostic criteria for RA, including morning stiffness, symmetric arthritis, and elevated rheumatoid factor levels, which must

persist for at least six weeks to confirm diagnosis [26]. Felson observed that RA patients benefit from long-term moderate to high-intensity exercise, which improves aerobic capacity, muscle strength, and slows bone mineral density loss, although there are concerns regarding the risks associated with intense physical activity. Piercarlo reviewed treatment options focusing on pain management and functional improvement, highlighting the widespread use of acetaminophen and NSAIDs for symptomatic relief [27].

FINDING AND DISCUSSIONS:

Table 1 shows the high serum level of MDA (0.875±0.425) in RA patients as compared to pre-treatment (0.613±0.401). Whereas there was statistically high significant plasma MDA activity in RA patients ($p<0.000$). We also found the actions of an important anti-oxidant GSH. Reduction of GSH (0.939±0.114) seen in RA patients in contrast to healthy persons (0.378±0.304). Statistically GSH was highly significant ($p<0.000$). Activity of catalase was higher (0.734±0.365) than in healthy individuals (3.299±0.184). Statistically catalase was highly significant ($p<0.000$). SOD quietly decreased in RA patients (0.580±0.367) as compared to pre-treatment (1.018±0.769). Superoxide dismutase significantly showed the increased actions ($p<0.000$).

TABLE: 1 PRE AND POST ANTIOXIDATIVE STATUS PROFILE IN RHEUMATOID ARTHRITIS PATIENTS

VARIABLES	Pre Treatment (N=60)	Post Treatment (N=60)	p<0.05
MDA	0.613±0.401	0.875±0.425	0.000
GSH	0.378±0.304	0.939±0.114	0.000
CATALASE	3.299±0.184	0.734±0.365	0.000
SOD	1.018±0.769	0.580±0.367	0.000

Values are articulated as mean ± standard deviation; n=60 considerably different from healthy individuals ($p<0.000$).

TABLE: 2 PRE AND POST VITAMIN PROFILE IN RHEUMATOID ARTHITIS PATIENTS

VARIABLES	Pre Treatment (N=60)	Post Treatment (N=60)	p<0.05
VITAMIN A	1.29±0.050	3.18±0.23	0.000
VITAMIN C	3.471±1.340	6.11±0.99	0.000
VITAMIN E	4.460±1.761	9.11±1.92	0.000

Mean values are shown as ± S.D; n=60 notably vary from normal individuals ($p<0.000$).

Table 2 depicts the non-enzymatic antioxidants (Vitamin A, C, and E) in plasma vitamin A in serum was critically low in RA patients (3.18±0.23) and (1.29±0.050) in healthy individuals. Statistically vitamin A was significant in RA patients ($p<0.000$). Vitamin C remarkably reduced in rheumatoid patients (6.11±0.99) as contrast to pretreatment persons (3.471±1.340). Statistical analysis shows increased significance ($p<0.000$) in diseased patients. Vitamin E in plasma of RA patients confirm drastically low (9.11±1.92) in RA patients whereas in healthy individuals was level (4.460±1.761). Reduced concentration of vitamin A, C, and E showed low anti-oxidant activities in diseased patients. Vitamins E low level is highly significant ($p<0.000$) as contrast to control.

TABLE : 3 PRE AND POST PROFILE OF DIFFERENT BIOMARKER IN RA PAETIENTS

VARIABLES	Pre Treatment (N=60)	Post Treatment (N=60)	p<0.05
AOPP	1.081±0.201	1.843±0.320	0.000
NITRIC OXIDE	10.346±3.328	0.191±0.190	0.000

Values designated as mean ± standard deviation, n= 60 appreciably dissimilar from fit individuals (p<0.000).

Table 3 illustrates advance oxidation protein products (AOPP) as biomarker of protein oxidation. Level of AOPP is amazingly high (1.843±0.320) in RA patients against pretreatment (1.081±0.201). AOPP was extremely significant (p<0.000) in While nitric oxide (NO) linked as mediator of inflammatory arthritis and noticed high level in RA patients (0.191±0.190) alongside healthy persons (10.346±3.328). NO concentration from RA patients was over twice (p<0.000) than that of healthy persons.

TABLE: 4 PRE AND POST ELECTROLYTES PROFILE IN RHEUMATOID ARTHRITIS PATIENTS

VARIABLES	Pre Treatment (N=60)	Post Treatment (N=60)	P<0.05
SODIUM (Na^+)	136.06±2.577	155.21±3.44	0.000
POTASSIUM (K^+)	4.222±0.559	6.11±1.22	0.000

Evaluate numbers are articulated as mean ± standard deviation, n=60 significantly different from healthy individuals (p<0.000).

Electrolytes profile of RA patients established in Table 4, sodium (Na^+) level was drastically enlarged in rheumatoid arthritis patients (155.21±3.44) as compared to pretreatment persons (136.06±2.577). Statistically Na^+ plasma level is highly significant (p<0.00) which was distinguish to normal. Potassium (K^+) was five times less in RA patients (6.11±1.22) against normal individuals (4.222±0.559). Statistically $K^{+\ is}$ highly significant in RA patients (p<0.00).

TABLE: 5 PRE AND POST RENAL PROFILE IN RHEUMATOID ARTHRITIS PATIENTS

VARIABLES	Pre Treatment (N=60)	Post Treatment (N=60)	P<0.05
UREA	27.59±8.27	44.31±4.29	0.000
CREATININE	0.81±0.59	2.19±0.36	0.000

Evaluate numbers are articulated as mean ± standard deviation, n=60 significantly different from healthy individuals (p<0.000).

Table 5 demonstrates Renal function tests (RFTs) as biomarker of renal health. Level of Urea is remarkably high (44.31±4.29) in RA patients against pre-treatment (27.59±8.27). Urea was extremely significant (p<0.000) in While creatinine noticed high level in RA patients (2.19±0.36) alongside healthy persons (0.81±0.59). Creatinine concentration from RA patients was over twice (p<0.000) than that of healthy persons.

TABLE: 6 PRE AND POST LIVER PROFILE IN RHEUMATOID ARTHRITIS PATIENTS

VARIABLES	Pre Treatment (N=60)	Post Treatment (N=60)	P<0.05
AST	33.91±25.386	51.29±3.11	0.000
ALT	35.469±40.423	47.48±2.98	0.000
ALP	147.142±56.245	159.82±9.28	0.000
TOTAL BILIRUBIN	0.593±0.230	1.02±0.09	0.000

Evaluate numbers are articulated as mean ± standard deviation, n=60 significantly different from healthy individuals (p<0.000).

Table 6 shows the high level of AST (51.29±3.11) in RA patients as compared to pre-treatment (33.91±25.386). Whereas there was statistically high significant AST activity in RA patients (p<0.000). We also found the actions of an important liver enzyme. Elevation of ALT (47.48±2.98) seen in RA patients in contrast to healthy persons (35.469±40.423). Statistically ALT was highly significant (p<0.000). Action of ALP was greater (159.82±9.28) than in healthy individuals (147.142±56.245). Statistically ALP was highly significant (p<0.000). Total bilirubin level quietly increased in RA patients (1.02±0.09) as compared to pre-treatment (0.593±0.230). Total Bilirubin significantly showed the increased actions (p<0.000).

TABLE: 7 PRE AND POST LIPID PROFILE IN RHEUMATOID ARTHRITIS PATIENTS

VARIABLES	Pre Treatment (N=60)	Post Treatment (N=60)	P<0.05
TOTAL CHOLESTEROL	178.367±23.431	146.36±6.11	0.000
TRIGLYCERIDES	109.346±21.649	89.48±5.68	0.000

Evaluate numbers are articulated as mean ± standard deviation, n=60 significantly different from healthy individuals (p<0.000).

Table 5 reveals Lipid profile as biomarker for cholesterol and triglycerides. Level of Cholesterol is remarkably low (146.36±6.11) in RA patients against pretreatment (178.367±23.431). Cholesterol was extremely significant (p<0.000) in While triglycerides noticed decreased level in RA patients (89.48±5.68) alongside healthy persons (109.346±21.649). Triglycerides concentration from RA patients was over (p<0.000) than that of healthy persons.

The pre- and post-treatment antioxidative status in rheumatoid arthritis (RA) patients showed significant changes. The serum level of Malondialdehyde (MDA), an indicator of oxidative stress, was notably elevated in post-treatment RA patients (0.875±0.425) compared to pretreatment values (0.613±0.401), with statistical significance (p<0.000). Conversely, the level of Glutathione (GSH), a critical antioxidant, increased significantly post-treatment (0.939±0.114) compared to pretreatment (0.378±0.304), indicating enhanced antioxidant defense (p<0.000). Catalase activity decreased substantially post-treatment (0.734±0.365) from pretreatment levels (3.299±0.184), and Superoxide Dismutase (SOD) levels also showed a marked reduction post-treatment (0.580±0.367 vs. 1.018±0.769), both with high statistical significance (p<0.000). These results demonstrate the oxidative imbalance in RA patients, characterized by increased oxidative stress markers like MDA and decreased anti oxidative enzyme activities, notably catalase and SOD. However, the significant increase in GSH post-treatment suggests some improvement in the antioxidant defense mechanism. The non-enzymatic antioxidants, including vitamins A, C, and E, exhibited substantial variation between pre- and post-treatment profiles in RA patients. [28] Vitamin A levels were significantly lower post-treatment (3.18±0.23) compared to pre-treatments (1.29±0.050), with statistical relevance (p<0.000). Similarly, Vitamin C levels showed an appreciable increase post-treatment (6.11±0.99) as

opposed to pre-treatments (3.471±1.340). A similar trend was observed in Vitamin E levels, which increased post-treatment (9.11±1.92) compared to pre-treatments (4.460±1.761), all with high statistical significance ($p<0.000$). [29] The results imply a significant depletion of vitamins A, C, and E in RA patients, suggesting compromised non-enzymatic antioxidant defense, which may contribute to the persistence of oxidative stress in RA patients. Advanced oxidation protein products (AOPP), a marker of protein oxidation, were significantly elevated in RA patient post-treatment (1.843±0.320) compared to pre-treatments (1.081±0.201), with statistical significance ($p<0.000$). Nitric oxide (NO), a mediator of inflammatory processes, was notably reduced post-treatment (0.191±0.190) as opposed to pre-treatments (10.346±3.328), indicating a decline in inflammatory activity post-treatment ($p<0.000$). The elevated AOPP levels and reduced NO concentration suggest that protein oxidation and inflammatory markers are critical components of RA pathophysiology, with treatments potentially mitigating these effects. [30] The electrolyte profile revealed significant changes in sodium (Na^+) and potassium (K^+) levels in RA patients. Post-treatment Na^+ levels (155.21±3.44) were markedly higher than pre-treatments values (136.06±2.577), while K^+ levels also showed a significant increase post-treatment (6.11±1.22) compared to pre-treatments (4.222±0.559), both with high statistical significance ($p<0.000$). The observed imbalance in Na^+ and K^+ levels highlights possible alterations in electrolyte homeostasis in RA patients, which could be attributed to inflammation or corticosteroid use. [31] Renal function tests (RFTs) indicated a significant rise in both urea and creatinine levels post-treatment. Urea levels increased from 27.59±8.27 to 44.31±4.29, and creatinine levels rose from 0.81±0.59 to 2.19±0.36, both highly statistically significant ($p<0.000$). These findings suggest impaired renal function in RA patients, possibly exacerbated by chronic inflammation or the effects of long-term treatment. The liver function markers, including AST, ALT, and ALP, were significantly elevated post-treatment. AST increased from 33.91±25.386 to 51.29±3.11, ALT from 35.469±40.423 to 47.48±2.98, and ALP from 147.142±56.245 to 159.82±9.28. Total bilirubin levels also rose post-treatment (1.02±0.09) compared to pre-treatments levels (0.593±0.230), all with high statistical significance ($p<0.000$). These results indicate potential liver dysfunction in RA patients, possibly due to disease progression or treatment-related side effects. The lipid profile revealed a significant decrease in total cholesterol and triglycerides post-treatment. [32] Total cholesterol dropped from 178.367±23.431 to 146.36±6.11, while triglycerides decreased from 109.346±21.649 to 89.48±5.68, both with statistical significance ($p<0.000$). This reduction in lipid levels suggests a potential therapeutic benefit of RA treatment in modulating lipid metabolism, which may lower cardiovascular risks associated with RA.

CONCLUSION AND FUTURE ASPECTS:

The study demonstrated significant alterations in antioxidative, vitamin, biomarker, electrolyte, renal, liver, and lipid profiles in rheumatoid arthritis (RA) patients. Elevated oxidative stress markers such as MDA and reduced activities of antioxidative enzymes like catalase and SOD underscore the oxidative imbalance in RA patients. Post-treatment increases in GSH and essential vitamins (A, C, E) suggest some restoration of antioxidant defense mechanisms. Biomarkers such as AOPP and nitric oxide showed marked changes, with elevated AOPP and reduced nitric oxide levels post-treatment, indicating a reduction in inflammatory processes. Electrolyte imbalances, notably in sodium and potassium, along with impaired renal function (elevated urea and creatinine), suggest a systemic impact of RA, which could be exacerbated by inflammation or treatment side effects. Liver function markers (AST, ALT, ALP) and lipid profiles revealed significant shifts, indicating potential hepatic dysfunction and improvements in lipid metabolism post-treatment.

Future research should focus on long-term follow-up of RA patients to assess the sustainability of antioxidative and anti-inflammatory improvements and to monitor potential side effects of prolonged treatment. Further exploration of the molecular mechanisms underlying oxidative stress and antioxidant imbalances in RA could lead to the development of targeted therapies aimed at restoring oxidative balance. Investigating new therapeutic approaches that can better manage the electrolyte, renal, and liver dysfunctions in RA patients would help improve overall treatment outcomes and

reduce long-term complications. Future studies should investigate the role of lipid metabolism in RA progression and whether lipid-lowering interventions could further reduce cardiovascular risks in these patients. Continued research into the use of biomarkers like AOPP and nitric oxide can aid in early diagnosis and tracking disease progression, leading to more personalized treatment strategies.

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