

**PREVALENCE OF ABNORMAL LIVER FUNCTION  
TESTS IN RHEUMATOID ARTHRITIS**

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## **DECLARATION**

This dissertation is my original work and has been presented as a prerequisite for a Master's degree to the Department of Clinical Medicine and Therapeutics, University of Nairobi, Kenya. It has not been presented for any degree to any other university.

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## **DEDICATION**

‘It is by standing on the shoulders of great men that I have been able to see far’,

To my supervisors who painstakingly sit with me and help refine my work,

To my husband who continually supports me,

To my children who give me the drive to go on,

To my colleagues who help to nourish my ideas,

To God for wisdom, strength and health,

I give thanks.

## **LIST OF ACRONYMS AND ABBREVIATIONS**

<b>ACR</b>	American College of Rheumatology
<b>AIH</b>	Autoimmune hepatitis
<b>ALP</b>	Alkaline Phosphatase
<b>ALT</b>	Alanine Aminotransferase/ Transaminase
<b>AST</b>	Aspartate Aminotransferase/ Transaminase
<b>BMI</b>	Body Mass Index
<b>DAS28</b>	Disease Activity Score - 28 joints
<b>DMARDs</b>	Disease Modifying Anti Rheumatic Drugs
<b>EDTA</b>	Ethylene diamine tetraacetic acid
<b>ELISA</b>	Enzyme linked immunosorbent assay
<b>ESR</b>	Erythrocyte Sedimentation Rate
<b>EULAR</b>	European League against Rheumatism
<b>GGT</b>	Gamma Glutamyl Transpeptidase
<b>HBV</b>	Hepatitis B Virus
<b>HCQS</b>	Hydroxychloroquine Sulphate
<b>HCV</b>	Hepatitis C Virus
<b>HIV</b>	Human Immunodeficiency Virus
<b>IU</b>	International Units
<b>IV</b>	Intravenous
<b>KNH</b>	Kenyatta National Hospital
<b>L</b>	Liter
<b>LEF</b>	Leflunomide
<b>LFTs</b>	Liver Function Tests
<b>Mls</b>	Milliliters

<b>MTX</b>	Methotrexate
<b>NSAIDs</b>	Non-steroidal anti-inflammatory drugs
<b>OCP</b>	Oral Contraceptive
<b>OR</b>	Odds Ratio
<b>PBC</b>	Primary Biliary Cirrhosis
<b>PI</b>	Principal Investigator
<b>RA</b>	Rheumatoid Arthritis
<b>SSA</b>	Sub-Saharan Africa
<b>ULN</b>	Upper Limit of Normal
<b>UoN</b>	University of Nairobi
<b>WHO</b>	World Health Organization
<b>Yr</b>	Year

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## **OPERATIONAL DEFINITIONS**

- Elevation of liver enzymes:** Any increase of liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) above the upper limit of the reference value provided by the laboratory
- Liver dysfunction:** Any abnormality of the liver function tests, that is reduction in albumin and total protein below the lower limit of the reference range, reversal of the albumin to globulin ratio and elevation of the total bilirubin, direct bilirubin, ALT, AST, ALP and GGT above the upper limit of the reference range provided by the laboratory
- Cholestatic liver disease:** The combination of elevated alkaline phosphatase, gamma glutamyl transferase and direct bilirubin
- Hepatocellular injury:** The combination of elevated enzymes alanine aminotransferase and/or aspartate aminotransferase

## **ABSTRACT**

### **BACKGROUND**

Rheumatoid arthritis is a systemic, chronic inflammatory polyarthritis that has high morbidity and mortality. Abnormal liver function tests in rheumatoid arthritis may result from the disease itself, drugs taken to treat the condition, injury caused by alcohol, infections such as viral hepatitis or other autoimmune disease affecting the liver. The burden of liver dysfunction in rheumatoid arthritis needs to be determined in our setting. This study set out to determine the prevalence of abnormal liver function tests in ambulatory patients diagnosed with rheumatoid arthritis at the rheumatology out-patient clinic, Kenyatta national hospital.

### **METHODOLOGY**

This was a cross-sectional study carried out in the rheumatology out-patient clinic of the Kenyatta national hospital. The study included 107 patients aged 18 years and over diagnosed with Rheumatoid arthritis. Consecutive sampling was used to recruit participants. After obtaining written and informed consent, participants' records were examined for previous liver function test results, medication used and duration of illness. Demographic data and medical history was collected using a pre-structured data capture form. A disease activity score using the DAS28-ESR scale was done. A brief physical exam to evaluate signs of liver disease and assess joint swelling and tenderness was then followed by collection of a blood sample to conduct liver function tests and erythrocyte sedimentation rate. Data was summarized using tables, pie charts, histograms bar charts and line graphs. Stata version 13 was used for data analysis. The prevalence of abnormal liver function tests was calculated as a percentage of the total liver function test results. Factors associated with liver dysfunction were determined using logistic regression.

### **RESULTS**

The overall prevalence of abnormal liver function tests in the study population was 57%. The most common abnormal liver function tests were direct bilirubin and alkaline phosphatase, which were elevated in 34.6% and 15% of the study population, respectively. Abnormal direct bilirubin was associated with longer duration of disease, adjusted odds ratio of 0.54 (0.34, 0.86) with a p value of 0.009 and with higher disease activity, adjusted OR 2.79(1.23, 6.25) with a p value of 0.014. Abnormal ALP was significantly associated with BMI with an

adjusted OR of 0.205 (0.074, 0.57), p value 0.002 as well as duration of disease, with an adjusted OR 1.14 (1.013, 1.29), p value 0.031.

## **CONCLUSION**

This study found the prevalence of liver dysfunction in patients with rheumatoid arthritis to be 57% and recommends regular monitoring of liver function tests in patients with rheumatoid arthritis.

# 1. INTRODUCTION

## 1.1 Rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic, chronic, progressive inflammatory disease characterized by symmetric joint polyarthritis that progresses to severe joint destruction (1,2). It is thought to be an autoimmune condition of unknown etiology. Rheumatoid arthritis is a major cause of disability and mortality. It is the most common rheumatologic condition, thought to affect at least 0.5-1% of the world's population (3). The actual burden of rheumatoid arthritis in Africa is unknown due to unavailable data on incidence and prevalence as well as limited diagnosis of the disease. It has however been projected that RA affects 4.3 million Africans, giving an estimated prevalence of 0.43% in 2010(4). Usenbo *et al* carried out a systematic review and meta-analysis of African studies, where he found that the prevalence of RA in urban settings ranged from 0.1% in Algeria, 0.6% in the DRC to 2.5% in South Africa. The prevalence in rural settings ranged from 0.07% in South Africa, 0.3% in Egypt to 0.4% in Lesotho(5).

The number of patients diagnosed to have RA at the Kenyatta national hospital (KNH) has been rising over the years, since 1979 when Bagg *et al* reported 76 patients with RA over a period of 18 months(6). In 2007, Owino *et al* reviewed 60 patients over 6 months at the KNH medical outpatient clinic(7). In 2012, Mbuthia *et al* was able to recruit 106 patients in his study over a similar duration (8). Later studies have reviewed similar numbers of patients within a shorter duration (9,10). RA is the most common rheumatologic condition and data from the rheumatology outpatient clinic at KNH showed 37.3% of patients had RA(11).

Diagnosis of RA is usually based on clinical findings and should be made early in the course of the disease so that treatment can be instituted before permanent joint deformities occur. Diagnosis is however difficult due to the non-specific signs and symptoms as the illness begins. The American College of Rheumatology/ European League against Rheumatology (ACR/EULAR) criteria 2010 aids the early diagnosis of RA (2). With increasing knowledge and awareness of rheumatologic conditions as well as access to revised clinical guidelines, we can now diagnose and treat the condition early.

Disease activity in rheumatoid arthritis refers to the severity of the client's symptoms and signs, including joint pain, stiffness and swelling. It reflects the underlying inflammatory process and may vary as a result of treatment. A Chinese cross-sectional study by Wang *et al*

among 486 RA patients found that absence of rheumatoid factor (RF) and antibodies to Cyclic Citrullinated Peptide (anti-CCP), treatment with Methotrexate (MTX) and Hydroxychloroquine Sulphate (HCQS) and a younger age were associated with lower disease activity (12).

Various disease activity indices are recommended by the American College of Rheumatology (ACR) to monitor clinical improvement in RA (1,13,14). The Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI) (15) and Disease Activity Score in 28 joints (DAS28) (16) have been used in clinical trials as well as practice to monitor for improvement and have recently been found to be equally effective (9). The DAS 28 tool using erythrocyte sedimentation rate (ESR) is validated to distinguish between low and high disease activity (16) and has been used in several studies carried out in Kenyatta National hospital (9,10,17,18).

Treatment is the greatest determinant of outcomes in patients with RA (19). Pharmacological treatment options in patients with RA include corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs) and biologic agents. Current treatment recommendations advocate the early use of DMARDs within three months of diagnosis (2,20). This is important in order to avert the joint destruction that is the sequelae of the disease. As a result, patients with RA face a lifetime of medication.

As a systemic illness, RA has many extra-articular manifestations and leads to co-morbidities, many of which have been studied in our local setting. The prevalence of pulmonary function abnormalities in patients with RA at KNH, Aga Khan and Mater hospitals was found to be 40% (21). Twenty six percent of RA patients were found to have peripheral arterial disease (17). Patients living with RA also have various hematological disorders, with 33% having anemia (18). Sanaa *et al* in 2015 found chronic kidney disease in 28% of RA patients at KNH(10). These co-morbidities have all been found to be associated with disease activity. Liver dysfunction has however not been investigated in our setting as an extra-articular manifestation in patients with RA.

Rheumatoid Arthritis can affect the liver in many ways (22). Liver dysfunction that arises in rheumatoid arthritis patients may arise from the disease itself, independent autoimmune disease, infections such as viral hepatitis or as a consequence of anti-inflammatory drugs such as disease modifying anti-rheumatic drugs (DMARDs). The most common DMARDs used in



treatment of RA in our setting are methotrexate and leflunomide, which are hepatotoxic. The risk of hepatotoxicity while on treatment with DMARDs may be increased in the presence of hepatitis or alcohol intake.

## **1.2 Abnormal liver function tests in rheumatoid arthritis**

The liver is a very important organ in the body with many complex and vital functions including metabolism of carbohydrates, protein and fat, synthesis of many proteins, secretion of bile and metabolism of drugs. The liver also plays a large role in the regulation of the coagulation system through the production of coagulation factors II, VII, IX and X as well as the anticoagulant proteins C and S. Biochemical tests of liver function include tests of liver injury, biosynthetic function and biliary metabolism.

Liver injury can be detected through the rise of liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and alkaline phosphatase. Destruction of hepatocytes results in seepage of these hepatic enzymes into the blood, where they can be detected. Elevated aminotransferases are found in almost all patients with liver disease and represents hepatocellular injury. Protein and albumin are synthesized by the liver and are important in establishing the synthetic function of the liver.

Liver function tests have been found to be abnormal in up to 50% of patients with RA and this has been shown to be associated with disease activity (23,24). Previous studies have noted histological changes in the liver of untreated RA patients such as fatty change, cellular necrosis, chronic passive congestion and gross atrophy (25–28). This may make it likely that RA in itself could cause liver dysfunction.

Abnormal liver function tests are an independent predictor of mortality (29). Due to high mortality from both rheumatoid arthritis as well as abnormal LFTs, such a population with RA could be at higher risk. This is especially so because currently we have limited ways of managing liver injury in our setting. It is therefore important for us to monitor for liver dysfunction in patients with rheumatoid arthritis.

## **2. LITERATURE REVIEW**

### **2.1 Tests for liver function**

It is a well-recognized fact that the liver is affected in RA, but the extent of liver dysfunction has not been conclusively determined. Over the years, various investigations have been done, ranging from biochemical tests and imaging to more invasive tests such as liver biopsies (23,25–28). Biochemical tests have been found to be sensitive enough to reveal liver pathology (30). Kremer *et al* in 1996 found that elevated AST levels in RA patients who had also undergone biopsy correlated with the higher histological grades (31).

#### **2.1.1 Liver biochemistry**

There are many biochemical tests used to check for liver function; Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin and bilirubin. All these tests reflect the different functions of the liver; secretion of anions (bilirubin), hepatocellular integrity (ALT and AST), synthesis (albumin and total protein) and free flow of bile (bilirubin and ALP) (32).

The enzymes ALT and AST are raised in cases of hepatocellular injury (33). Their function in gluconeogenesis is to catalyze the transfer of amino groups from alanine and aspartic acid to ketoglutaric acid to produce pyruvic acid and oxaloacetic acid respectively. Alanine aminotransferase is more specific to the liver than AST (30).

Alkaline phosphatase increases the breakdown of organic phosphate esters. Though its precise function is not yet known, it is thought to down-regulate the secretory activities of the intrahepatic biliary epithelium (33,34). ALP rises due to increased synthesis and release into the circulation. ALP is also produced in the bones, and can be raised in both physiological and pathological states. Pregnant women in their third trimester as well as adolescents have a high ALP level due to the increase in metabolism. In biliary obstruction, levels rise up to two days later and take several days to return to normal because of a long half-life. ALP may be elevated in infiltrative disease and malignancies, even in the absence of bone or liver involvement (35). ALP is important in recognizing cholestatic disease (30). Distinguishing bone from liver ALP necessitates electrophoretic separation and checking 5' nucleotidase or GGT, which rise in liver but not bone disease.

Gamma glutamyl transferase (GGT) facilitates transfer of the gamma-glutamyl group from gamma-glutamyl peptides such as glutathione to other peptides and to L-amino acids. It therefore aids in transport of amino acids. This enzyme is found in hepatocytes and biliary epithelial cells and is sensitive but non-specific for hepatobiliary disease (36). Gamma glutamyl transferase is thought to correlate with ALP in determining cholestatic disease. It is also raised in pancreatic disease, myocardial infarction, renal failure, chronic obstructive pulmonary disease, diabetes and alcoholism (36). Isolated elevations of GGT may signify alcohol liver disease and alcohol abuse, due to induced microsomal production or a possible alcohol-induced leakage of the enzyme from the cells (29,36,37). Anti-epileptic drugs like phenytoin, carbamazepine, and barbiturates can also cause mild elevation in GGT.

Synthesis of all proteins except gamma globulins takes place in the liver. Measurement of total proteins would therefore be indicative of liver function. However, the decrease in proteins synthesized by liver is compensated by increased synthesis of gamma globulins by plasma cells.

This limits the use of total protein in determining liver function.

The liver synthesizes and secretes about 10 g of albumin daily. With progression of liver disease, serum albumin levels fall due to decreased synthesis. Factors such as nutritional status, catabolism, hormonal factors, and urinary and gastrointestinal tract losses affect albumin levels and should be considered when interpreting low albumin levels (35). Low albumin suggests chronic liver disease such as cirrhosis or cancer, while normal albumin suggests acute liver disease such as viral hepatitis or choledocholithiasis. The ratio of serum albumin to globulin gives a better idea of liver function and should normally be more than 1.5.

Bilirubin is produced from breakdown of red blood cells. Unconjugated bilirubin, after binding to albumin, is transported to the liver and conjugated to bilirubin glucuronide, which is then secreted into bile and the gut. Serum bilirubin is normally unconjugated, reflecting balanced production and hepatobiliary excretion. Unconjugated bilirubin increases in hemolysis, ineffective erythropoiesis and hematoma resorption. Conjugated hyperbilirubinaemia is elevated in parenchymal liver disease or biliary obstruction (35).

Reference values of liver biochemistry tests, derived from the Kenyatta national hospital renal laboratory are shown in table 1;

**Table 1: Reference values of liver function tests (Kenyatta national hospital)**

<b>Liver function test</b>	<b>Normal Value</b>
ALT	5-40 IU/L
AST	3-35IU/L
ALP	40-150IU/L
GGT	0-50IU/L
Albumin	37-60g/L
Protein	60-80g/L
Total Bilirubin	1.7-20.6 $\mu$ mol/
Direct Bilirubin	0-8.6 $\mu$ mol/L

### **2.1.2 Excretion tests of liver function**

Excretion tests such as the indocyanine green clearance, <sup>14</sup>C-aminopyrine breath test, antipyrine clearance, galactose elimination capacity, bromsulphthalein and <sup>13</sup>C-caffeine breath test are mainly used in research settings to evaluate liver function. The indocyanine green clearance test remains the only test for establishing true global liver function (38).

### **2.1.3 Use of histology and imaging in assessing liver function**

Histology is important in demonstrating parenchymal liver disease. Currently, with widespread use of liver biochemistry and imaging, it is not standard practice to use liver biopsies to check liver function.

Ultrasonography, magnetic resonance imaging (MRI) and computerized tomography (CT) scan are useful in assessing post-operative patients. These imaging tests have the advantage of displaying the spatial distribution of liver function, as compared to liver biochemistry. Nuclear medicine scans, planar scintigraphy can also be used to assess liver function (38).

## **2.2 Prevalence of abnormal liver function tests in rheumatoid arthritis**

Various studies have used different methods to check liver function in RA patients over the years, ranging from biochemical tests to histology. Currently, there is increasing use of biochemical tests to assess liver function. Earlier studies carried out before the use of DMARDs assessed liver function in patients with untreated RA and even then, the ‘rheumatologic liver’ was a topic of interest(26,27,39,40). Currently, more patients living

with RA are on DMARDs and more studies have been prospectively assessing the effect of drugs on the liver function of RA patients(41–45). Comparison of both older studies and recent studies in RA patients is important in elucidating what may cause abnormal liver function tests.

Patients who have active RA commonly have hepato-splenomegaly and derangements of liver biochemistry (22). Elevated alkaline phosphatase (ALP) has been reported in up to 50% of patients with RA (23,24,39).

In 1955, Lefkowitz *et al* cited contradictory information regarding the involvement of the liver in RA and thus reviewed liver function of 86 RA patients. They used serum bilirubin, ALP, bromsulphthalein, cholesterol, cephalin flocculation, total protein, albumin, thymol turbidity, and prothrombin as well as histology to assess liver function. They found that the most common abnormalities were elevated serum globulin and low albumin, followed by abnormalities in cholesterol, cephalin flocculation, and thymol turbidity. They also demonstrated a clear relationship between disease activity and the abnormality in liver function, with 65% of stage 1 RA, 87% of stage 2 RA, 95% of stage 3 and 100% of stage 4 RA having abnormal results. Biopsies of the liver showed definite abnormal morphological changes only in two out of the fifteen specimen reviewed (39).

Cockel *et al* carried out a case-control study among 100 RA patients and 100 matched controls and showed that 26% of the patients with RA had raised ALP, but found normal levels of ALT and bilirubin despite the evidence of liver disease (24). Webb *et al* in 1975 carried out a study looking at markers of liver dysfunction in 216 patients with RA, 32 patients with sjogren's disease and 27 patients with the sicca syndrome, and compared results with 289 patients with osteoarthritis or a form of negative spondyloarthropathy. They conducted clinical examination for liver disease and reviewed serum ALP, bilirubin, ALT, AST, total protein, albumin and globulin, bromsulphthalein (BSP) excretion test, mitochondrial antibody, smooth muscle antibody and histology. They found significantly more hepatomegaly (10%), higher ALP (18%) and abnormal BSP in patients with RA as compared to other groups, which correlated with disease activity (23). ALT was elevated in 19% and AST in 0.5% of patients with RA.

In a study by Kendall *et al* that compared 15 RA patients with raised ALP and those with normal ALP, elevation of ALP was found to be associated with disease activity (46). The correlation of the elevated ALP with elevated 5- Nucleotidase (5-NT) levels confirmed the source of ALP was from the liver. Fernandez *et al* in a survey of 100 RA patients, found that 45 had biochemical evidence of liver disease, mostly elevations in ALP and/ or GGT (47). Approximately 18% had elevations of both ALP and GGT, suggesting hepatobiliary disease.

Liver biopsies of patients with RA have shown portal tract inflammation, fibrosis, fatty liver and cirrhosis. A review of liver histology findings at autopsy of 182 patients with RA before the era of methotrexate treatment found that fatty change (24 cases) or passive congestion (44 cases) were listed as their most common hepatic finding. In 15 cases, no abnormal hepatic histology was seen on the autopsy. Fibrotic changes were found in 11% (25). Mills *et al* carried out liver biopsies in a series of 31 rheumatoid arthritis patients with clinical and/or biochemical evidence of hepatic dysfunction. Four of the 31 (13%) were found to have definable chronic liver disease, normal hepatic histology or non-specific reactive changes being found in the remainder. In many patients, the hepatic abnormality remains functional and unexplained (28). Cases of acute liver failure, however, have also been reported.

Table 2 summarizes studies demonstrating abnormal liver function tests in patients who were not yet on treatment with DMARDs, therefore reflecting the effect of the disease.

**Table 2: Studies done showing prevalence of liver dysfunction in patients with rheumatoid arthritis not on treatment**

<b>Investigator and Study design</b>	<b>Number of Patients</b>	<b>Liver function tests</b>	<b>Results</b>
Lefkowitz <i>et al</i> , 1955 Cross-sectional	86	Bilirubin, albumin, globulin	Low albumin , high globulin
Webb <i>et al</i> , 1975 Cross-sectional	216	ALP, bilirubin, ALT, AST, total proteins, albumin and globulin,	Elevated ALP
Cockel <i>et al</i> , 1971 Case-control	100	ALP, albumin, globulin	Elevated ALP-26%, Normal ALT, bilirubin  Low albumin, high globulin
Fernandes <i>et al</i> , 1979	100	ALP, GGT	45%- Abnormal LFTs  18%-raised ALP

Cross-sectional

+GGT

## **2.3 Causes of abnormal liver function tests in rheumatoid arthritis**

There are various manifestations of liver disease in RA patients. Abnormal liver function may be due to the disease itself, drugs used in treatment such as NSAIDs and DMARDs, infections by Hepatitis B or C viruses or autoimmune diseases such as Primary biliary cirrhosis or autoimmune hepatitis.

### **2.3.1 Rheumatoid arthritis as a cause of abnormal liver function tests**

Liver injury is not directly implicated as an extra-articular feature of RA, though elevations of serum enzymes do occur. Studies done by Lefkovitz *et al* in 1955 suggested a relationship between the severity of rheumatoid arthritis and abnormal liver function tests. Elevation of the serum globulin and depression of the serum albumin were the most frequent liver function test abnormalities, with histology showing non-significant alteration (39). Cockel *et al* also showed a correlation between the disease activity in patients with RA with the elevated liver enzymes (24).

### **2.3.2 Drugs that may cause abnormal liver function tests in patients with rheumatoid arthritis**

Any drug may cause a dysfunction in liver biochemical tests. Common causes include non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, statins, antiepileptic drugs, antituberculous drugs or even herbal preparations. Drug-induced liver injury is common in RA patients, especially with high use of analgesics like NSAIDs and DMARDs such as methotrexate (48). Serial measurements of ALT and AST have been endorsed to identify patients at risk of developing methotrexate-related liver fibrosis.

In a retrospective survey of the large Arthritis, Rheumatism, and Aging Medical Information System (ARAMIS) database, with almost 2600 RA patients, liver toxicity was found in about 5% of patients, more commonly in patients on salicylates (7.6%), sulindac (8.3%) and methotrexate (9.5%). Combination therapy of methotrexate and salicylates was responsible for a lot of the hepatic enzyme abnormalities and led to withdrawal of methotrexate (49). In a cross-sectional descriptive study at KNH from 2005-2006, less than half of RA patients were on DMARDs with 25% on NSAIDs alone (7). Recent studies have shown that 87% of RA patients are on DMARDs (9,10).



### **2.3.2.1 Effect of methotrexate on the liver in rheumatoid arthritis**

Methotrexate (MTX) is an anti-metabolite and anti-folate drug that works by inhibiting dihydrofolic acid reductase, purine and thymidilic acid synthesis, thus interfering with Deoxy ribonucleic acid (DNA) synthesis, repair and cellular replication. In RA, it inhibits proliferation of inflammatory cells implicated in the progression of disease. It is thus indicated in the management of severe, active disease, has been shown to have cardiovascular benefits and is thought to improve mortality (50).

MTX is metabolized by the liver and is known to cause frequent but mild elevations in serum aminotransferase. In 10-50% of patients, ALT and AST are elevated to less than twice the upper limit of normal and this may be self-limiting. Five percent of patients may have higher levels of liver enzymes, which may or may not respond to stopping the drug or modifying the dose. Long duration of treatment has been implicated in the development of chronic liver injury and is linked to fatty liver disease, fibrosis and cirrhosis.

Liver injury is thought to occur due to accumulation of MTX and depletion of folate. MTX causes an increase in adenosine levels which is activated to cyclic adenosine monophosphate, which is immunosuppressive (41,51). Liver injury secondary to MTX occurs in female patients, those with a higher body mass index (BMI) and those who have had a longer duration of therapy (43,52). Elevation of ALT and/or AST above the upper limit of normal before starting treatment has also been linked to methotrexate hepatotoxicity. Older patients are presumed to have a higher likelihood of liver fibrosis caused by drugs due to reduced kidney function and frequent adverse effects of concomitant medications (53). In a study by Kent *et al*, methotrexate-induced hepatotoxicity was more likely in patients who did not get folate supplementation, had a high BMI and untreated hyperlipidemia (54).

Folic acid reduces the hepatotoxicity caused by MTX and is now commonly supplemented in patients on the drug (55,56). In a retrospective study of 1224 Dutch patients in 2003, Hoekstra *et al* found that toxic effects were the major factor that led to stopping MTX in 50% of RA patients 9 years of treatment. Supplementation of folic acid was associated with continued use of MTX in patients (57).

Severe liver disease has been found in less than 3% of RA patients on low-dose MTX. Phillips *et al* found that 3 of 134 RA patients on MTX developed clinically significant

hepatic dysfunction and showed histologic evidence of severe liver disease (fibrosis and cirrhosis). Factors identified in these patients that may have been linked to liver toxicity included diabetes, congestive heart failure and Felty's syndrome. In the patient group that received a post-MTX liver biopsy, pulmonary fibrosis and obesity were significantly associated with hepatic fibrosis/cirrhosis (58).

Beyeler *et al*, in a longitudinal study of RA patients on low-dose MTX treatment, found elevated enzymes and reduced quantitative function in 14%. Biopsies carried out on the patients with liver dysfunction showed fibrosis and cirrhosis, which did not seem to correlate with the weekly MTX drug dosage, alcohol use or age (44). In a meta-analysis by Conway *et al*, methotrexate was associated with an increased risk of total adverse liver events, as well as minor and major liver enzyme abnormalities. Patients treated with MTX were not at increased risk of liver failure, cirrhosis or death (45).

A recent review on the use of methotrexate in RA by Genga *et al* makes note of the fact that low-dose methotrexate has been found to be safe, but stresses on the need for monitoring of LFTs and investigation of any liver dysfunctions(59).

Table 3 summarizes studies carried out on effect of low-dose methotrexate on the liver.

**Table 3: Studies showing effect of methotrexate on liver function of patients with rheumatoid arthritis**

<b>Study</b>	<b>Number of patients</b>	<b>Follow-up duration</b>	<b>Results</b>
Weinblatt ME <i>et al</i> , 1990 Prospective	281	36wks	ALT elevations >2XULN in 24%
Scully CJ <i>et al</i> , 1991 Prospective	124	5yrs	Elevated serum enzymes in 70%
Schnabel A , 1994 Prospective	168	1 yr	ALT or AST elevations in 38% on 15 mg/week and 44% on 25 mg/week
Hoekstra <i>et al</i> , 2003 Prospective RCT	411	48wks	ALT elevations >3X ULN in 26% on placebo vs. 4% on folate
Tilling L <i>et al</i> , 2006 Prospective	550	13yrs	ALT or AST elevations >3 times ULN in 7.5% RA patients
Salliot <i>et al</i> , 2009 Systematic review	3463	3 yrs	73% - at least one adverse event 18.5% - a liver related event 20% - at least one elevation in liver enzymes, 13% >2X ULN
Amital <i>et al</i> , 2009 Retrospective	119	1 year	45% - at least 1 abnormal liver test

### **2.3.2.2 Effect of leflunomide on the liver in rheumatoid arthritis**

The mechanism of action of leflunomide (LEF) is not well-understood, but it is thought that its metabolite inhibits synthesis of pyrimidine nucleotides, which reduces the proliferation of T-cells. It is metabolized by the liver and gastrointestinal mucosa and excreted in both urine and faeces. Adverse effects include hepatitis, jaundice/ cholestasis, hepatic failure or necrosis. Treatment interruption of leflunomide is recommended if ALT rises to more than three times the upper limit of normal. In case of leflunomide-induced rise in ALT, the patient should be treated with cholestyramine and ALT should be monitored weekly until normal. Use of both MTX and LEF is not advised in elevation of liver enzymes of more than twice the upper limit of normal and in case of positive Hepatitis B and C serology (2,20).

Treatment of RA with MTX and LEF in combination has been associated with even more hepatotoxic effects. Curtis *et al* in 2010 evaluated liver enzymes in patients with RA who were started on DMARDs. ALT or AST elevations were found in 17% of patients on LEF alone, 22% on MTX alone, and 31% on both. They found hepatotoxicity, defined as enzyme elevations higher than twice the ULN in 1%-2% of patients on MTX or LEF monotherapy and in 5% of patients on combination therapy. After multivariate analysis, the MTX and LEF combination was associated with a greater risk of hepatotoxicity. Patients with history of liver disease and alcohol use had a higher risk of elevation of liver enzymes (42). This however differs from results of a Brazilian study conducted on 71 RA patients which found no statistical difference in aminotransferase elevations between those on MTX monotherapy (11.1%) and those with MTX and LEF combination therapy (11.5%) (60).

Katchamart *et al* carried out a systematic review of randomized trials comparing MTX alone and in combination with other non-biological DMARDs to evaluate drug efficacy and toxicity in 1624 adults with RA. Combination therapy resulted in more withdrawals due to adverse reactions than monotherapy, but the differences were significant only for cyclosporine and azathioprine (61). However, case reports do exist in the literature of acute liver failure and death resulting from combination DMARD hepatotoxicity (62). More trials are needed in evaluating the long-term effects of combination DMARDs on the liver since results are inconclusive.

Table 4 summarizes the effect of MTX in combination with leflunomide on the liver.

**Table 4: Studies of the effect of combination DMARDs on liver function in patients with rheumatoid arthritis**

Study	Results
Curtis <i>et al</i> , 2010 Retrospective	LEF-17% ALT/AST elevation MTX-22% ALT/AST elevation MTX+LEF-31% ALT/AST elevation
Rodriguez Alves <i>et al</i> , 2011 Retrospective	MTX-11.1% ALT/AST elevation MTX+LEF-11.5% ALT/AST elevation
Gupta R <i>et al</i> , 2011	MTX+LEF -22% ALT/ AST elevation

### **2.3.2.3 Hepatotoxicity of other DMARDs in patients with rheumatoid arthritis**

Sulfasalazine is a combination of salicylic acid and sulfapyridine and is a second-line drug in the management of RA (2). Sulfasalazine is effective in slowing disease progression in RA, but its use and retention may be limited by its adverse effects, which include hepatotoxicity. A study comparing the safety and retention on combination treatment with DMARDs found that sulfasalazine was associated with lower retention in care and was involved in 49% of the adverse reactions (63). Jobanputra *et al* found that 0.4% of patients on sulfasalazine were likely to have serious liver disease (64).

Azathioprine is used as a second-line DMARD in RA (2,20). Azathioprine is reported to cause acute drug-induced liver injury. Nodular regenerative hyperplasia and veno-occlusive disease can also result from the chronic use of Azathioprine (48). A systematic review found that the use of Azathioprine is associated with high withdrawals from treatment due to hepatotoxicity (65).

Biologic DMARDs have been implicated as a cause of hepatotoxicity. A case-control analysis of hepatic events in two American cohorts of 41,885 RA patients on DMARDs, found higher rates of abnormal liver function tests with etanercept and infliximab when compared with methotrexate and leflunomide(66). Compared to other DMARDs however, MTX is still thought to cause more hepatotoxicity (67) .

#### **2.3.2.4 Analgesics causing abnormal liver function tests in rheumatoid arthritis**

Acetaminophen is commonly taken as an over-the-counter drug. It can cause elevation of aminotransferase among healthy adults even when taken in recommended doses. In a randomized controlled trial carried out among healthy volunteers taking acetaminophen (4 g daily for 14 days), about 20 percent experienced an ALT elevation more than five times the ULN (compared with 3 percent taking placebo). An increase of 1 to 2 times the ULN was observed in 50 to 70 percent of patients (68).

Salicylates and NSAIDs have also been found to cause acute elevations in liver enzymes (48). A study by Rodriguez found that patients with RA had 11-fold higher risk of developing NSAID-induced hepatotoxicity. Concomitant use of NSAIDs with other hepatotoxic medication can increase the risk even further (69). Data correlating liver enzyme elevations and anti-rheumatic medication found higher levels with the use of methotrexate and salicylates as compared to salicylates alone (49).

#### **2.3.2.5 Other drugs commonly used in rheumatoid arthritis that cause abnormal liver function tests**

Antituberculous medication has been implicated as a causal agent for liver dysfunction in RA patients. Isoniazid (INH) is commonly used in our setting for prophylaxis as well as treatment of tuberculosis. Van hoof *et al* found that 4 of the 8 RA patients put on INH got hepatotoxicity, which resolved on stopping. None of the hepatotoxicity was clinically apparent. A study by Bourré-Tessier *et al* investigated the risk of hepatotoxicity in rheumatic patients taking INH while on DMARDs and found 24% of the patients had abnormal LFTs during INH treatment compared to 12.1 % prior to INH (70).

#### **2.3.3 Effect of Alcohol on the liver in rheumatoid arthritis**

Excess alcohol consumption, more than 100g/day (8 drinks) for 2 or more decades, leads to a range of hepatic manifestations such as alcoholic fatty liver disease, hepatitis and cirrhosis. Quantity and frequency of alcohol intake is associated with risk of alcoholic liver disease (71). Patients may present with jaundice, fever, anorexia and tender hepatomegaly. Abnormal liver function tests such as elevated transaminases of about 300IU/L, with an AST to ALT ratio of two or greater, elevated bilirubin, GGT and International normalized ratio can occur.

The lower level of ALT relative to AST is thought to be due to low levels of pyridoxal 5-phosphate in alcoholics. The enzyme is a co-factor in the activity of ALT and a lower value reflects inability to increase ALT levels(72–74).

Despite the effect of alcohol on the liver, it has been thought to be protective against development of RA (75). A case-control study in the UK that reviewed 873 RA patients and 1004 healthy controls from 1996 to 2006 found that increased alcohol intake was associated with up to 4 times less risk of developing RA as well as reduced disease severity. The patients with RA did not take more alcohol than the controls, and were also less likely to drink alcohol while on DMARDs. It has long been believed that RA patients may take alcohol for the analgesic effect. This has however not been proven (76). Current theories propose that RA patients may reduce alcohol intake due to concurrent intake of NSAIDs and DMARDs or that alcohol may indeed be protective (77). Alcohol would require consideration in patients with RA due to treatment that can be used in patients with liver damage due to alcohol (78). The risk of hepatotoxicity with MTX use is increased in the presence of alcohol use (79).

#### **2.3.4 Infections that contribute to liver dysfunction in patients with rheumatoid arthritis**

Patients with RA may also have hepatitis, which may result in elevations of liver enzymes. Hepatitis B and C viruses are the most common causes of hepatitis in our setting. Kenya is thought to have intermediate endemicity of HBV, with a prevalence of 2 to 8% (80).

Seventy percent of patients infected with Hepatitis B virus (HBV) may present with a sub-clinical hepatitis, while 30 percent develop clinical hepatitis with jaundice (81). Case reports of RA patients co-infected with Hepatitis B demonstrate that patients may have a severe clinical presentation, leading to acute liver failure, especially when put on treatment with DMARDs. This effect is seen especially on withdrawing MTX, resulting in sudden reactivation of the immune system which then attacks the virus-infected cells (82–85). Hepatitis B reactivation causing severe hepatitis can also occur in patients put on DMARDs (86).

Laboratory testing during the acute phase of HBV reveals elevations in the concentration of ALT and AST; values up to 1000 to 2000 IU/L are typically seen during the acute phase with ALT being higher than AST. The serum bilirubin concentration may be normal in patients

with sub-clinical hepatitis. In patients who recover, normalization of serum aminotransferases usually occurs within one to four months. A persistent elevation of serum ALT for more than six months would indicate a progression to chronic hepatitis.

Maillefert *et al* found that the prevalence of Hepatitis C in patients with RA was 0.65%, which was equal to that of the general population (87). Most patients who have hepatitis C are asymptomatic and have a clinically mild course. They may have jaundice, malaise, nausea, and right upper quadrant pain. Infection with Hepatitis C virus (HCV) can result in acute or chronic hepatitis (88). Following infection, serum aminotransferases are elevated 6 to 12 weeks following exposure. Serum ALT levels vary and a study of 44 patients with HCV found the mean ALT level was 885 U/L (+/- 554 U/L)(89). The ALT level may normalize in some patients, even in the presence of the infection. Resultant fulminant hepatic failure is rare, but may occur with concomitant HBV infection. ELISA tests for HCV are positive within 8 weeks of exposure.

Hepatitis C infection can also present with rheumatologic manifestations. A case study of 19 patients in Washington, USA found that Hepatitis C viral infection can present with rheumatic manifestations indistinguishable from RA. The patients were referred due to polyarthritis, polyarthralgia and positive rheumatoid factor and were later found to have Hepatitis C infection. The predominant clinical findings include palmar tenosynovitis, small joint synovitis and carpal tunnel syndrome. Risk factors for infection such as blood transfusions and intravenous drug abuse or a history of hepatitis or jaundice should be included in the history of present illness of any patient with acute or chronic polyarthritis or unexplained positive rheumatoid factor (RF). It is recommended that in such patients, GGT, serologic studies for Hepatitis C and other tests appropriate for chronic liver disease should be performed(90).

Mok *et al* reviewed the safety of DMARDs in 29 RA patients with chronic viral hepatitis; 23 with hepatitis B virus and 6 with Hepatitis C virus. ALT elevations were found in 41% on hydroxychloroquine, 30% on methotrexate and 14% on gold vs. 14% of 94 control patients without viral hepatitis (91). The use of DMARDs in patients with RA and chronic viral hepatitis was found to result in hepatotoxicity, with a synergistic effect even among non-hepatotoxic drugs. Current recommendations provide that physicians screen for viral hepatitis before starting treatment with DMARDs (20).



We do not know the current prevalence of HBV and HCV in the local population with RA. However, a question arising is whether it is safe to use methotrexate as a first-line treatment for RA in our setting, with the suspected high prevalence of HBV and HCV infection (92).

### **2.3.5 Autoimmune liver disease as a cause of liver dysfunction in rheumatoid arthritis**

Autoimmune hepatitis (AIH) is a generally unresolving inflammation of the liver of unknown etiology. Environmental triggers, failure of immune tolerance mechanisms and genetic predisposition work to induce T cell-mediated immune attack upon liver antigens, leading to a progressive necro-inflammatory and fibrotic process in the liver (93,94). Autoimmune liver disease has a variable presentation and patients may be asymptomatic or present with acute liver disease (95).

Two different studies by Whaley *et al* and Webb *et al* that used anti-mitochondrial antibodies to evaluate prevalence of autoimmune disease in RA found a low prevalence of 0.66% and 0.97% (23,96). This was higher than in patients with Sjogren's syndrome or combined RA and sjogren's syndrome. The latter study also found low prevalence of smooth muscle antibody. Patients with RA do not appear to have marked prevalence of concomitant autoimmune liver disease.

## **2.4 The impact of liver dysfunction in rheumatoid arthritis**

Abnormal liver function even in the general population has been noted to correlate with increased mortality. A study by Ruhl *et al* evaluated the effect of an elevated GGT and ALT on overall mortality. They found that an elevated GGT was associated with a modestly higher mortality from all causes (Hazard Ratio (HR), 1.5; 95% CI, 1.2-1.8), liver disease, cancer, and diabetes, while an elevated ALT was associated with an increase in liver-related mortality (HR, 8.2; 95% CI, 2.1-31.9) (29).

Persistent elevation of liver enzymes has been shown to correlate with increased burden of fibrosis assessed by liver biopsy, as shown by several studies by Kremer *et al* among RA patients who were on treatment with DMARDs (31,97,98). Rheumatoid arthritis patients who have elevated liver enzymes are at a further risk of developing fibrosis and would benefit from close monitoring.

Liver dysfunction plays a major role in determining which medication the physician can prescribe for the RA patient. A lot of the DMARDs used in RA, including methotrexate and leflunomide are metabolized by the liver. Hepatotoxicity limits the type of drug, combinations as well as dosages that can be used to control disease activity in RA. Monitoring liver enzymes would be important to improve compliance in RA patients and to avoid combinations that may worsen hepatotoxicity. The duration and compliance of patients on treatment with DMARDs would be affected by the hepatotoxicity as well. In a 9-yr longitudinal follow up of RA patients on DMARDs, 14% of patients stopped treatment due to hepatotoxicity (52).

Infections with HBV and HCV preclude the use of DMARDs in RA patients (2,20). It is therefore critical to evaluate the prevalence of abnormal liver function in this population, so as to ensure optimal liver function and use of drugs to control disease activity in RA patients.

## **2.5 Management of abnormal liver function tests in rheumatoid arthritis**

The most important aspect of managing liver dysfunction in RA patients is to diagnose the cause. If the cause is drugs, the drug should be withdrawn and the liver function monitored. Any infections diagnosed should be treated. Further choice of treatment would depend on the clinical presentation of the patient. Kremer *et al* suggests that pretreatment biopsy need only be carried out in patients with persistently elevated liver enzymes or risk factors for liver disease. Regular liver test monitoring at 4-8 week intervals and repeat liver biopsy can be done if AST elevations occur [at least 5] or albumin levels fall. Methotrexate can be discontinued if AST elevations persist and one is unable to do liver biopsy (2,20,97).

Modification of the methotrexate dose may also reduce hepatotoxicity. Salaffi *et al* followed up 51 RA patients on weekly methotrexate for 3 years and found that 14% developed AST and ALT elevations, which resolved spontaneously or on reducing the dose of the drug (99).

## **2.6 Study Problem**

Rheumatoid arthritis is a systemic autoimmune disease that is life-long and debilitating. With increasing awareness and knowledge of the disease, more patients are being diagnosed early and started on treatment. Effective treatment modalities include NSAIDs and DMARDs which have hepatotoxic effects.

The liver has previously been overlooked as a target organ for disease in the rheumatologic setting. We do not know the current burden of liver dysfunction in patients with rheumatoid arthritis in our setting, which may lead to sub-optimal care in these patients. Some drugs such as methotrexate and sulfasalazine, which form the mainstay of DMARD therapy, cannot be used in patients with liver disease.

## **2.7 Study justification**

This study serves as a baseline survey on the magnitude of liver dysfunction in patients with Rheumatoid arthritis. It is important to establish associated factors for liver dysfunction that clinicians should look out for. The findings of this study will therefore improve monitoring for liver disease among patients with RA and improve quality of care given.

## **2.8 Research Questions**

1. What is the burden of liver dysfunction among patients with rheumatoid arthritis attending the Kenyatta National Hospital rheumatology clinic?

## **2.9 Objectives**

### **2.9.1 Broad objective**

To evaluate the burden of abnormal liver function among patients with rheumatoid arthritis attending the Kenyatta National Hospital rheumatology clinic.

### **2.9.2 Specific objectives**

1. To determine the prevalence of abnormal liver function tests among patients with rheumatoid arthritis attending the Kenyatta National Hospital rheumatology clinic
2. To determine the association of abnormal liver function tests with disease activity using Disease activity score in 28 joints-ESR score (DAS28-ESR) among patients with rheumatoid arthritis

### **Secondary objective**

1. To determine the association of abnormal liver function tests with medication (NSAIDs, DMARDs and corticosteroids) , clinical and socio-demographic characteristics of patients with rheumatoid arthritis attending the rheumatology clinic at KNH

## **3.0 STUDY METHODS**

### **3.1 Study design**

This was a cross-sectional descriptive survey among patients with rheumatoid arthritis at the outpatient rheumatology clinic of Kenyatta national hospital.

### **3.2 Study Site**

This study was carried out in the outpatient rheumatology clinic of Kenyatta national hospital, Nairobi, Kenya. Kenyatta national hospital is a national referral and teaching hospital and receives many patients who are referred from other facilities in the country for further investigation and management. It is currently the only public hospital with a specialized rheumatology clinic. The rheumatology clinic is held weekly on Thursday afternoons and approximately 70 patients are seen per clinic. The clinic is run by consultant rheumatologists and physicians from Kenyatta national hospital and the University of Nairobi, assisted by post-graduate residents from the Pediatric and Internal medicine departments. First-time patients are seen by the consultants and a diagnosis is made. On subsequent visits, the patients are attended to by a resident, in consultation with the consultants.

### **3.3 Study population**

The study population was patients diagnosed with rheumatoid arthritis attending the KNH rheumatology clinic from February to April 2016.

### **3.4 Inclusion and exclusion criteria**

#### **Inclusion criteria**

Patients were included if they:

- i. had a diagnosis of rheumatoid arthritis according to 2010 ACR-EULAR criteria
- ii. had an age of at least 18 years
- iii. gave written informed consent
- iv. attended the KNH rheumatology clinic in the year 2016

## Exclusion criteria

Patients were excluded if they;

- i. were suspected to have mixed connective tissue disease or juvenile rheumatoid arthritis
- ii. did not meet the inclusion criteria

## 3.5 Case definition of liver dysfunction

Patients were reported as having abnormal liver function tests if they had any elevations in the liver enzymes ALT, AST, ALP, GGT above the upper limit of the reference range, a rise in total or direct bilirubin above the upper limit and reduction of the albumin and protein levels below the lower limit of the reference range. High changes in enzyme levels were read as multiples of the upper limit of the reference range and were taken to reflect the severity of liver dysfunction.

Patterns of liver injury were determined as well. Hepatocellular injury was determined from elevations of ALT and/or AST and cholestatic liver disease was determined from the elevations of ALP, bilirubin and GGT.

## 3.6 Sample size calculation

The Fisher et.al. 1998 formula was used to calculate sample size as follows;

$$n_0 = \frac{Z^2 \times p \times (1 - p)}{d^2} = \frac{1.96^2 \times 0.45 \times (1 - 0.45)}{0.05^2} = 380.3 \approx 380$$

Where  $n_0$  is the initial sample size, Z is the abbisca of the normal distribution under 5% error estimate (1.96), p is the prevalence of elevation in LFTs (45%) and d is the standard error allowed (5%).

Since the total patients with RA in KNH were approximately 146 (according to KNH records) which was <10,000, then the finite population correction factor was applied to determine the final sample size given by:

$$n = \frac{n_0}{1 + \frac{n_0 - 1}{N}} = \frac{380}{1 + \frac{380 - 1}{146}} = 105.8 \approx 106$$

The study therefore targeted **106 patients** as the final sample size.

### **3.7 Participant screening, sampling and recruitment**

The principal investigator (PI) together with trained study assistants who were clinical officers reviewed files of patients attending the rheumatology out-patient clinic. The files of patients who met the criteria were selected. Universal sampling was used to recruit participants. The patients were then given the relevant information about the study and those who were willing to participate and give written informed consent (Appendix 1) were recruited. Once written informed consent was obtained, the PI or study assistants then administered a questionnaire and carried out appropriate investigations.

### **3.8 Study variables**

#### **Independent variables**

Independent variables included socio-demographic and clinical variables;

- Age- recorded as number of years from reported or documented date of birth
- Sex- categorized as male or female and was determined by observation of participant phenotypic characteristics
- Level of education- recorded as the highest level of education the participant had acquired
- Marital status- categorized as single, married, divorced or widowed and was obtained from the participant
- Treatment modality- defined as drug used, dosage and duration of use. Drugs were classified under steroid, NSAID and DMARD. It was determined by the participant's report and confirmed from the participant's records.
- Disease duration from diagnosis- determined from the participant's report or documented date of diagnosis
- Alcohol- a detailed history of significant alcohol intake was taken and read as number of days when alcohol was taken over the last 1 month for participants who drink alcohol. The duration of alcohol intake as well as the type and amount of alcohol taken was recorded. For participants who were currently not taking alcohol, duration since they had stopped was recorded.
- Smoking – smoking history was classified as never smoked, previously smoked or current smoker. Smoking duration was measured in pack years.

## **Dependent variables**

Dependent variables were liver function test values and the DAS28-ESR score. The liver function tests included ALT, AST, GGT, ALP, direct bilirubin, total Protein and albumin.

The DAS 28-ESR score is graded from 0-10. It is calculated using a standardized and validated DAS 28 form (Appendix 5), which involves an assessment of the participant's swollen and tender joints, global health assessment and ESR measurements. The values of the DAS-28 were then calculated for each patient, using a DAS28 calculator that takes account of all 4 measurements. Thereafter, DAS28-ESR scores were categorized into remission (less than 2.6), low disease activity (2.6 to 3.2), moderate (3.2 to 5.1) and high disease activity (more than 5.1) so as to describe disease activity of the population.

## **3.9 Data collection**

### **3.9.1 Clinical methods**

The PI and the study assistant then took a brief history and carried out a clinical examination, as per the study data capture form (Appendix 3). The history included components of the DAS28 score, such as how the patient thought they were doing on a scale of 0 to 100 (patient global assessment) and how many of the pre-defined joints currently had pain. The clinical examination entailed assessment for jaundice, liver size and splenomegaly as well as palpation of the joints for swelling and tenderness as per the DAS28 score. The participant's file was reviewed to obtain information on disease duration, medication and duration of treatment, previous liver function tests, Hepatitis B, Hepatitis C and HIV results as well as history of alcohol intake. This information was then recorded in the data capture form for later analysis.

### **3.9.2 Laboratory methods**

#### **Specimen collection, transport and storage**

The PI or study assistant collected 5mls of blood from each participant. The samples were drawn aseptically from the cubital fossa, after swabbing the site with an alcohol swab. Three milliliters (mls) were placed in a plain vacuttainer for LFTs while 2mls were placed in an EDTA vacuttainer. Vacuttainers were provided by the KNH laboratory. Cooler boxes with ice packs at approximately 4° C, (2-8° C) were used for temporary storage and to facilitate

transport of samples to the laboratory. Samples were delivered to the KNH renal laboratory and University of Nairobi Hematology department laboratory at the end of the day's collection. These were analyzed upon delivery to the laboratory.

### **Specimen analysis**

Liver function tests analysis was carried out in the KNH renal laboratory using the Biolys Superior 50i which is an automatic biochemistry analyzer. It was able to analyze the following parameters; ALT, AST, GGT, ALP, albumin, total protein, total and direct bilirubin using the manufacturer's protocol.

Liver dysfunction in this population was diagnosed as per the patient's physical examination and laboratory parameters using the ALT, AST, GGT, total protein and albumin, total and direct bilirubin. Reference values for these tests from the laboratory were used to determine dysfunction. Any increase above the upper limit of normal for the enzymes was read as an abnormal liver function test. Any decrease of albumin or total protein below the lower limit of normal was read as an abnormal liver function test.

Determination of ESR was done at the Department of Hematology laboratory, University of Nairobi. It was done using the Wintrobe method. In this method, non-hemolysed blood collected in an Ethylene diamine tetraacetic acid (EDTA) vacuttainer is placed in a tube and the rate of fall of red blood cells is measured in millimeters (mm) after one hour.

### **Quality assurance**

Standard operating procedures for collection and transport of specimen were followed, with timely delivery to the laboratories to minimize pre-analytical errors. Specimen for LFTs and ESR were analyzed at the KNH renal laboratory and UoN Department of Hematology laboratory respectively. Quality control for the biochemistry analyzer was performed daily at the renal unit laboratory and whenever discrepant results were received. Quality control measures were provided by the company servicing the machine. Both laboratories undergo internal and external quality control measures and are run by qualified laboratory technologists.



### **3.10 Study administration**

The responsibility of the PI was to inform patients with RA about the study, recruit those willing to participate and obtain informed consent. The study assistants worked with the PI to ensure that data was collected efficiently, on time and that it was recorded accurately.

All recorded data was verified by the PI, who also ensured that all relevant forms were completed. The supervisors offered guidance to the PI throughout the process. The statistician offered guidance during proposal development, data entry, analysis and presentation of the final statistical analysis.

### **3.11 Data management**

Data was collected by the PI and the study assistants. All filled consent and data collection forms were stored in a lockable cabinet, accessed only by the principal investigator and the study clinician. Data was entered weekly into a password-protected Microsoft Excel database managed by the statistician. Once data entry was complete, entries in the database were compared to manual records to ensure accuracy. Checks were performed for data completion and inconsistencies were manually resolved with a review of manual records before data analysis.

### **3.12 Data analysis**

Categorical data was summarized as frequencies and percentages. Continuous data was summarized using measures of central tendency and dispersion (mean, standard deviation).

The prevalence of abnormal liver function tests was calculated as number of abnormal liver function test results as a percentage of the total number of liver function test results. Association where the predictor and outcome were categorical was demonstrated using chi-square tests and odds ratios whereas Analysis of variance (ANOVA) tests were used to show relationships between categorical outcomes and continuous predictors. Where both predictor and outcome were continuous, Pearson correlation coefficients were used to characterize the association. Linear regression analysis was used to identify independent predictors of outcomes.

Model building was done using a forward step-wise approach to identify the most parsimonious model. The level of significance was set at 0.05.

Stata version 13 was used for data analysis.

### **3.13 Ethical considerations**

The study was undertaken only after approval by the Department of Clinical Medicine and Therapeutics, University of Nairobi and the KNH/ UoN Ethics and Research Committee (ERC), Research Approval number P771/12/2015(Appendix 6).

Written informed consent was obtained (Appendix 1 and 2). The objectives and purposes of the study were clearly explained to eligible participants in a language suitable to them prior to enrollment into the study and they were allowed to ask questions and seek clarification. Only participants who gave written informed consent were enrolled.

Personal unique study numbers were assigned to the patients. The information obtained was kept confidential. Participants were allowed to withdraw from the study without discrimination. Only blood samples intended for study were drawn and thereafter discarded after analysis. All raw data will be destroyed within a year of completion of the study.

Copies of all laboratory results were availed in the patients' files, to assist clinicians in care of the patients. We also highlighted abnormal liver function test results for the healthcare givers to take action. Clinicians were also advised that liver function tests are non-specific and may not point to specific liver disease. They would therefore need to use clinical judgment in evaluating the patients further.

### **3.14 Results dissemination policy**

The results of this study will be disseminated to the primary health-care providers, the department, and all relevant decision-making bodies.

## 4. RESULTS

### 4.1 Participant recruitment and reasons for exclusion

The study ran from 14<sup>th</sup> February to 21<sup>st</sup> April 2016 and 107 patients were enrolled into the study. Three RA patients declined to give consent, citing reasons such as lack of time, having been involved in previous studies and not having got results back as well as a reluctance to have samples taken. All 107 patients enrolled into the study willingly gave written informed consent.

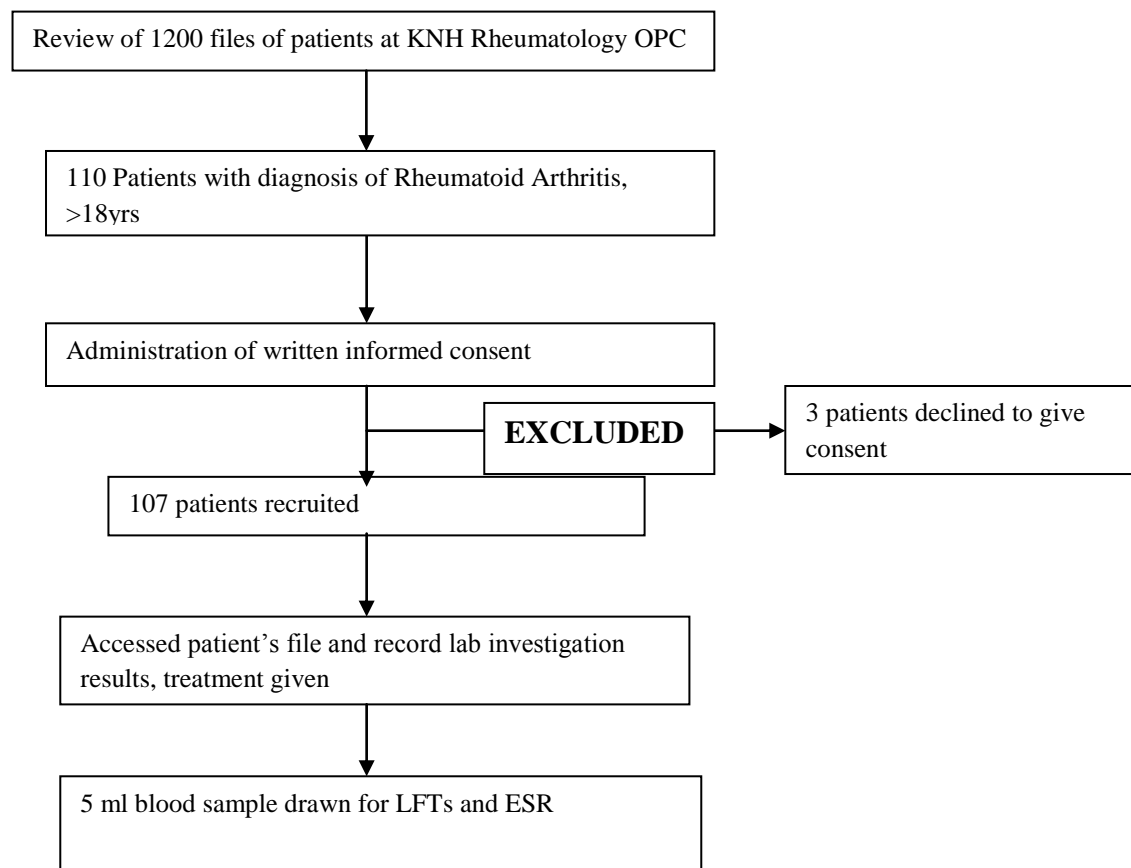


Figure 1: Study flow chart

## **4.2 Socio-demographic characteristics of the study participants**

The socio-demographic characteristics of the study participants are summarized in table 5.

The study population was 107 patients, and 90.7% of the participants were females. The male to female ratio was 1:9.7. The age of the study participants was not normally distributed and was negatively skewed. We did not therefore describe the mean. The median age was 50 years, ranging from 18 to 81 years.

Sixty five percent of participants had some form of employment and were engaged in business, formal employment or farming. Thirty five percent of them were either unemployed, had domestic duties or were in learning institutions. Most of the participants were drawn from areas surrounding Nairobi, with 34.3% being from Nairobi, 46% from Central and 12% from lower Eastern. This may reflect the catchment area of the hospital. However, 9.5 % came from far regions. Fifty eight percent of the participants were married and were thus assumed to have some form of social support. About 5% of participants had no formal education while forty three percent of participants had attained at least a primary school level of education; 32.7% had secondary level of education and 19.6% had a tertiary level of education.

### **Alcohol and smoking history**

One of the participants reported being a current smoker and one reported having stopped several years prior. Most were non-smokers. Alcohol intake in RA patients was slightly higher, with approximately 7.5% reporting current alcohol intake and 15% having stopped taking alcohol.

**Table 5: Socio-demographic characteristics of the study population**

Variable			
Age	Median 50 (35, 62)	Min 18	Max 81
		n	%
<b>Gender</b>	Male	10	9.3
	Female	97	90.7
<b>Occupation</b>	Unemployed, housewife, student	40	37.4
	Employed	18	16.8
	Self-employed, farmer	47	43.9
	Retired	2	1.9
<b>Residence</b>	Nairobi	37	34.6
	Central	46	43.0
	Lower Eastern	13	12.1
	Upper Eastern	2	1.9
	Rift valley	6	5.6
	Western, Nyanza	2	1.9
	Coast	1	.9
<b>Education level</b>	None	5	4.7
	Primary	46	43.0
	Secondary	35	32.7
	Tertiary	21	19.6
<b>Marital status</b>	Single, Separated	24	22.4
	Married	62	57.9
	Widowed	21	19.6

### 4.3 Clinical characteristics of the study population

Most of the study population (55%) had been diagnosed to have Rheumatoid arthritis for more than five years. Only 11% had been diagnosed one year prior to the study period. Rheumatoid factor was positive in 80% of the study population, negative in 8% and was unavailable in the files for 12%. Anti CCP was positive in 47% and negative in 7% of participants. Few participants had anti CCP results available in the files (55%).

Table 6 summarizes clinical characteristics of the study population.

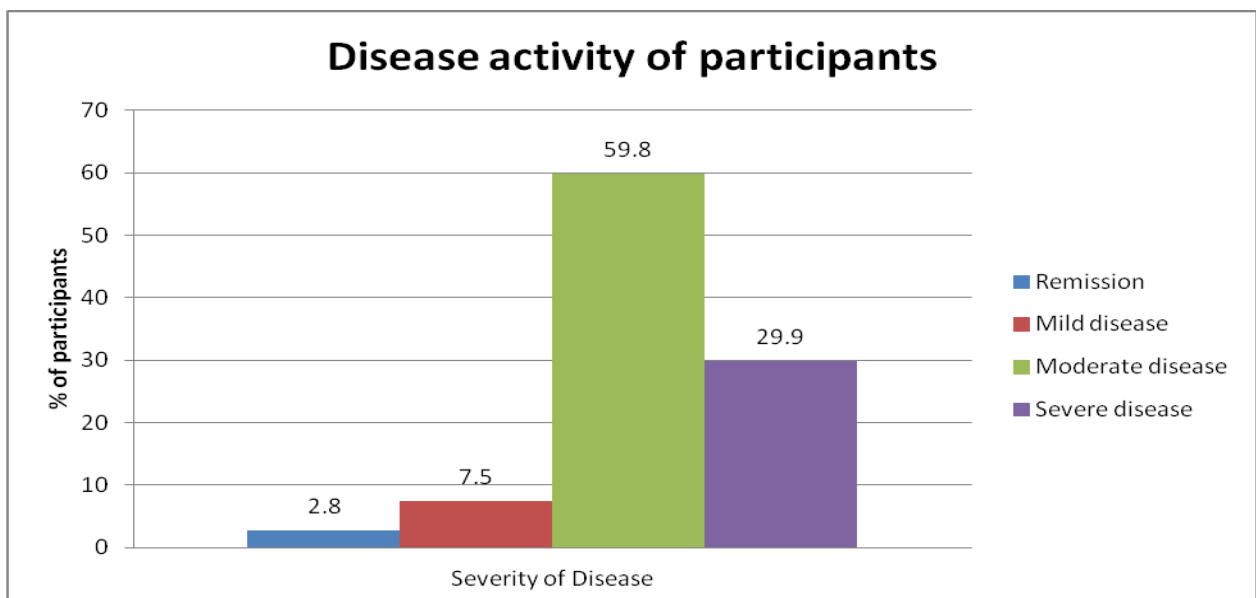
**Table 6: Clinical characteristics of the study population**

Variable		n	%
Duration diagnosed with RA	<1 year	12	11.2
	1-5 years	36	33.6
	>5 years	59	55.1
Rheumatoid factor available	Negative	14	13.7
	Positive	86	84.3
	Unavailable	7	6.5
Anti CCP available	Negative	7	6.5
	Positive	50	46.7
	Unavailable	50	46.7

### 4.3.1 Disease activity of study participants

Active disease was present in 97% of the study participants and only 3% were in remission. Most of the patients, 61%, had moderate disease activity at the time of the study, despite some being on multiple DMARDs.

The following bar chart shows the distribution of study participants according to disease activity.



**Figure 2: Disease activity of study participants**

## Independent factors associated with disease activity

Use of HCQS was found to be associated with a high DAS score, reflecting poor control of disease. Patients who were on HCQS were 1.7 times more likely to have poor disease control (OR=1.7, 1.14-2.55, p=0.011). A high BMI was also found to be associated with more severe disease (OR=1.06, 1.02-1.11, p=0.002).

### 4.3.2 Medication history of study participants

Use of NSAIDs among the study population was prevalent, with at least 80% currently using NSAIDs. Seventy six participants (86.4%) were using both analgesics and DMARDs.

Most of the participants (86%) were on DMARDs at the time of the study, as confirmed from patient files and previous prescriptions used. The most common DMARD used was methotrexate (MTX); 60% of the participants were on this drug. Forty one percent of the study participants were using HCQS. Leflunomide was used by 23% and sulfasalazine by 8% of participants. Forty six percent of participants were using steroids; mainly prednisone at variable doses. Only 1.9% of participants were on a steroid alone for RA control. There was no study participant using biologic agents for RA control. Eleven percent (11%) of participants were taking a combination of methotrexate and leflunomide, 3.7% on methotrexate and sulfasalazine, as well as 2.8% on sulfasalazine and leflunomide. Use of herbal medication was surprisingly high at 22.4%.

Table 7 summarizes medication use in the study population.

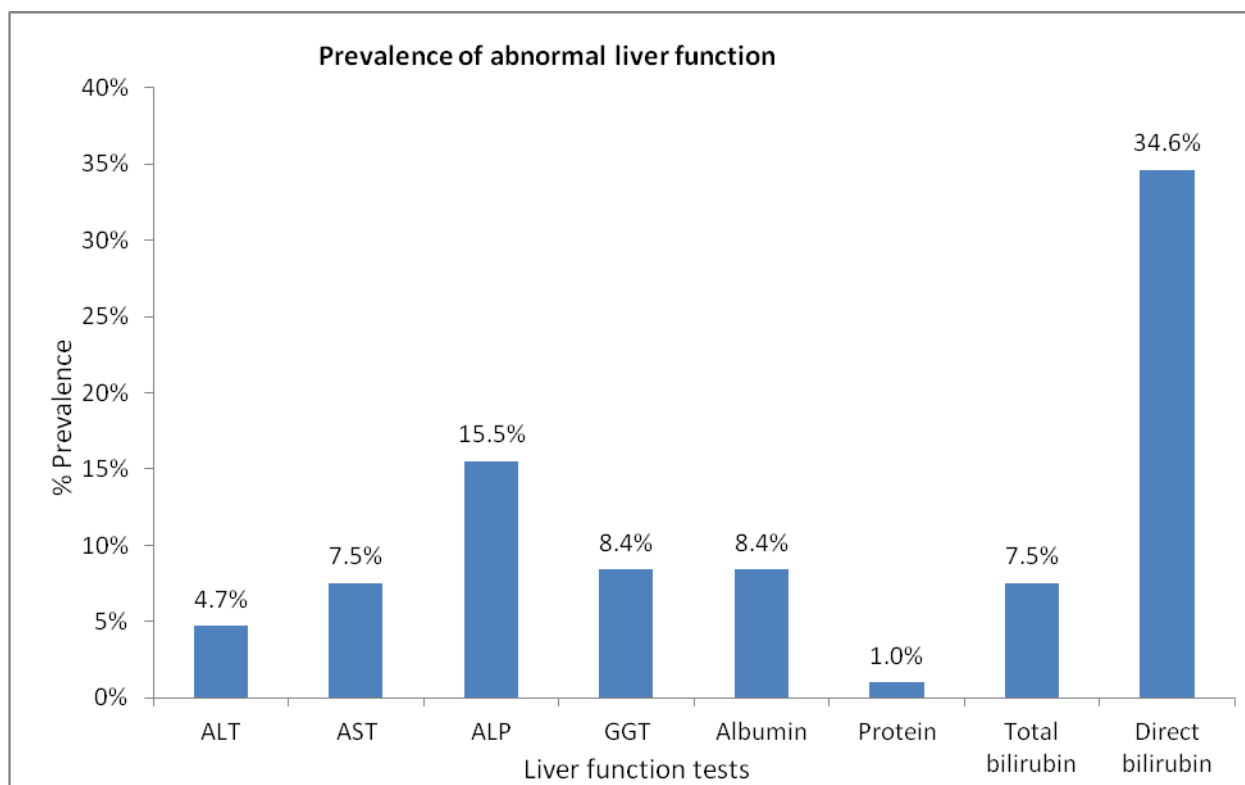
**Table 7: Medication use among participants**

Drug	Use n (%)	Duration		
		<3 months n (%)	3 months - 1 year n (%)	> 1 year n (%)
NSAID	86 (80.4)	2 (2.3)	23 (26.1)	63 (71.6)
Methotrexate	65 (60.7)	1 (1.5)	10 (15.4)	54 (83.1)
HCQS (mg/day)	44 (41.1)	1 (2.2)	6 (13.3)	38 (84.4)
Steroids - prednisone	49 (45.8)	2 (4)	7 (14)	41 (82)
Sulfasalazine (grams/day)	8 (7.5)	2 (22.2)	0 (0)	7 (77.8)
Leflunomide	25 (23.4)	4 (15.4)	4 (15.4)	18 (69.2)
Statin	4 (3.7)	2 (40)	0 (0)	3 (60)
Antiepileptic drugs	2 (1.9)	1 (50)	0 (0)	1 (50)
Oral Contraceptive Pills	8 (7.6)	1 (12.5)	2 (25)	5 (62.5)
Herbal medication	24 (22.4)	16 (15)	3 (2.8)	5 (4.7)

## 4.4 Liver function tests in the study population

### Prevalence of abnormal liver function tests in rheumatoid arthritis

Among the RA patients, 61 (56%) had at least 1 abnormal LFT result. The most common abnormality was elevated direct Bilirubin, which was found in 34.6% of participants. ALP was elevated in 15.5%. Abnormal GGT and albumin values were found in 8.4 % of participants. AST and total bilirubin were elevated in 7.5 % of participants. Less than 1% of patients had low protein. Figure 3 illustrates the prevalence of abnormal LFTs among study participants.



**Figure 3: Prevalence of abnormal liver function tests in rheumatoid arthritis**

Participants who had abnormality in both ALP and GGT were only 6 (5.4%). Participants who had elevations of more than twice the upper limit of normal range for the enzymes were fewer, with 5.6% having abnormal direct bilirubin, 1.9% with abnormal AST, 1.9% with abnormal GGT, 1% with abnormal ALP and total bilirubin and 0.9% with abnormal ALT.



#### 4.4.1 Correlates of elevated direct bilirubin

With longer disease duration, participants were almost three times more likely to have elevated direct bilirubin OR 2.79 (1.23, 6.25), with a p value of 0.014. Though not significantly associated, being female and having a high education level seemed to prevent development of elevated direct bilirubin. Drugs such as HCQS, especially at high doses were also protective against abnormal direct bilirubin. Due to the small number, further analysis of study participants on these drugs was not carried out. Tables 8 and 9 detail the associations, showing the ORs and adjusted ORs .

**Table 8: Logistic regression for elevated direct bilirubin**

<b>Variable</b>	<b>OR (95% CI)</b>	<b>P val</b>
Age	1.01 (0.98, 1.03)	0.66
<b>Gender</b>	0.313(0.082, 1.190)	<b>0.088</b>
Occupation	0.963(0.629, 1.473)	0.862
Residence	0.979 (0.710, 1.349)	0.896
<b>Education level</b>	0.659(0.402, 1.081)	<b>0.099</b>
Marital status	1.003(0.543, 1.856)	0.991
<b>RA Duration</b>	2.144 (1.099, 4.181)	<b>0.025</b>
BMI	0.996 (0.92, 1.078)	0.912
<b>DAS28</b>	0.593 (0.396, 0.888)	<b>0.011</b>
Rheumatoid factor	1.268(0.361, 4.459)	0.711
Anti CCP	1.452(0.255, 8.267)	0.675
Previous LFT	0.727(0.212, 2.5)	0.613
Co morbidities	1.26(0.68, 2.31)	0.46
Analgesic use	0.676(0.245, 1.862)	0.449
NSAID Duration	1.493(0.588, 3.7921)	0.4
DMARD started	1.538(0.453, 5.215)	0.49
MTX	1.095(0.483, 2.483)	0.83
MTX Duration	1.65 (0.441, 6.24)	0.45
MTX Dose	1.076 (0.973, 1.191)	0.155
HCQS	1.143(0.51, 2.56)	0.746
HCQS Duration	1.605(0.337, 7.641)	0.552

HCQS Dose	0.997 (0.992, 1.003)	0.375
Prednisone	1.969(0.878, 4.413)	0.1
Prednisone Duration	1.79 (0.506, 6.325)	0.36
Prednisone Dose	1.05 (0.938, 1.174)	0.397
SSZ	1.147(0.258, 5.091)	0.857
SSZ Duration	0.632 (0.126, 3.165)	0.572
SSZ Dose	0.999(0.998, 1.000)	0.31
Alcohol duration	1.015(0.896, 1.149)	0.82
Alcohol stopped	0.97(0.82-1.146)	0.718
Leflunomide	1.085 (0.426, 2.765)	0.865
Leflunomide duration	2.21(0.56, 8.712)	0.257
Leflunomide dose	0.981(0.913, 1.054)	0.609
OCP	0.253(0.03, 2.14)	0.207
Alcohol	0.918 (0.466, 1.812)	0.806
Alcohol days taken	0.474(0.89, 2.812)	0.41
Herbal medication use	1.481(0.583, 4.068)	0.409
Herbal med duration	1.5 (0.553, 4.068)	0.426

\*\*\*Unable to assess Hepatitis B Vaccine, Folate, Statin use, OCP duration, Smoking, smoking duration due to small number of variables

**Table 9: Adjusted odds ratios for abnormal direct bilirubin**

<b>Variable</b>	<b>Crude OR (95% CI)</b>	<b>P val</b>	<b>Adjusted OR</b>	<b>P-value</b>
<b>DAS28</b>	0.593 (0.396, 0.888)	<b>0.011</b>	0.54 (0.34, 0.86)	<b>0.009</b>
<b>RA Duration</b>	2.144 (1.099, 4.181)	<b>0.025</b>	2.79(1.23, 6.25)	<b>0.014</b>
<b>Gender</b>	0.313(0.082, 1.190)	<b>0.088</b>	0.603(0.43,1.03)	<b>0.054</b>
Education level	0.659(0.402, 1.081)	0.099	0.6(.33, 1.09)	0.096

#### 4.4.2 Correlates of elevated alkaline phosphatase

Fifteen percent of the study population had elevated ALP. Notably, abnormal ALP was significantly associated with BMI with an adjusted OR of 0.205 (0.074, 0.57), p value 0.002 as well as duration of disease, with an adjusted OR 1.14 (1.013, 1.29), p value 0.031.

Other independent predictors of elevated ALP included occupation and use of oral contraceptives. Having an occupation appeared to be protective for elevated ALP, OR 0.41 (0.16, 1.01) with a p value of 0.05. However, use of OCP was up to 22 times predictive for elevated ALP OR 22.3 (1.72, 290.12), p value 0.018. Having been started on any DMARD and drugs such as HCQS, methotrexate and prednisone were noted to be protective against development of abnormal LFTs. Due to small numbers however, this was not further analyzed.

**Table 10: Logistic regression for abnormal alkaline phosphatase**

<b>Variable</b>	<b>OR (95% CI)</b>	<b>P val</b>
Age	0.974(0.943, 1.007)	0.123
Gender	1.731(0.24, 14.69)	0.615
<b>Occupation</b>	<b>0.434(0.227, 0.83)</b>	<b>0.012</b>
Residence	1.052 (0.700, 1.581)	0.807
Education level	1.183(0.626, 2.237)	0.6
Marital status	0.913(0.407, 2.05)	0.826
<b>BMI</b>	<b>1.168(1.051,1.298)</b>	<b>0.004</b>
<b>RA Duration</b>	<b>0.247 (0.108, 0.564)</b>	<b>0.001</b>
DAS28	1.037(0.64, 1.682)	0.881
Rheumatoid factor	0.93(0.183, 4.726)	0.93
<b>AntiCCP</b>	<b>0.1(0.017, 0.705)</b>	<b>0.02</b>
Co-morbidity	1.66 (0.847, 3.27)	0.14
Previous LFT	0.987(0.197, 4.942)	0.987
Analgesic use	3.913(0.484, 31.626)	0.201
NSAID Duration	0.502(0.17, 1.488)	0.214
<b>Prednisone</b>	<b>0.215(0.057, 0.809)</b>	<b>0.023</b>

Prednisone Dose	0.973 (0.744, 1.273)	0.844
DMARD started	0.304(0.079, 1.166)	0.083
MTX	0.526(0.18, 1.542)	0.242
<b>MTX Duration</b>	<b>0.243 (0.061, 0.971)</b>	<b>0.045</b>
MTX Dose	0.829 (0.683,1.008)	0.06
Folate	0.122(0.007, 2.19)	0.154
Leflunomide	1.048 (0.305, 3.6)	0.94
Lefludur	1.74(0.285, 10.64)	0.549
Lefludose	0.984 (0.89, 1.088)	0.759
<b>HCQS</b>	<b>0.16(0.034, 0.748)</b>	<b>0.02</b>
<b>HCQS Duration</b>	<b>0.105(0.011, 1.014)</b>	<b>0.05</b>
HCQS Dose	1.001 (0.988, 1.014)	0.836
SSZ	1.929(0.353, 10.535)	0.448
SSZ Dose	1.001(0.999, 1.004)	0.165
Alcohol	0.643(0.219, 1.888)	0.42
Alcohol duration	0.796(0.427, 1.481)	0.471
Alcohol stopped	0.674(0.355, 1.279)	0.227
Statin	1.867 (0.181, 19.164)	0.599
<b>OCP</b>	<b>9.577(1.458, 62.906)</b>	<b>0.019</b>
Herbal	1.742(0.537, 5.657)	0.355
Smoking	1.833 (0.281, 11.946)	0.527

\*\* Herbal duration, Alcohol days taken, OCP Duration, SSZ Duration, Prednisone Duration not assessed due to low frequency of samples

**Table 11: Adjusted odds ratios for abnormal alkaline phosphatase**

Variable	OR (95% CI)	P val	Adjusted OR	Adjusted p value
<b>BMI</b>	1.168(1.051,1.298)	<b>0.004</b>	0.205 (0.074, 0.57)	<b>0.002</b>
MTX Dose	0.829 (0.683,1.008)	0.06	-	-
<b>RA Duration</b>	0.247 (0.108, 0.564)	<b>0.001</b>	1.14 (1.013, 1.29)	<b>0.031</b>
<b>Occupation</b>	0.434(0.227, 0.83)	<b>0.012</b>	0.405(0.16, 1.01)	<b>0.053</b>
Anti CCP	0.1(0.017, 0.705)	<b>0.02</b>	-	-
DMARD started	0.304(0.079, 1.166)	<b>0.083</b>	-	-
MTX Duration	0.243 (0.061, 0.971)	<b>0.045</b>	-	-
Folate	0.122(0.007, 2.19)	0.154	-	-
HCQS	0.16(0.034, 0.748)	<b>0.02</b>	-	-
HCQS Duration	0.105(0.011, 1.014)	<b>0.05</b>	-	-
Prednisone	0.215(0.057, 0.809)	<b>0.023</b>	-	-
<b>OCP</b>	9.577(1.458, 62.906)	<b>0.019</b>	22.3 (1.72, 290.12)	<b>0.018</b>

Liver function tests done 6 months prior to the study period were available for only 13 participants and of these; there was no significant change in the paired differences. This is shown in table 12.

Only one patient reported having had vaccination for Hepatitis B. Very few patients, 16%, had results for Hepatitis B and C investigations in the file, which were all negative. None of the patients had clinical liver disease, as evidenced by jaundice, hepatomegaly or splenomegaly. Average BMI in the population was 25.7, ranging from 16.4 to 43.5.

**Table 12: Change in value of liver function tests**

LFT value	Initial: n (%)			Final: n (%)			Paired diff
	Below Range	Within range	Above range	Below range	Within range	Above range	P value
ALT		12 (92.3)	1 (7.7)		102 (95.3)	5 (4.7)	1.0
AST		11 (84.6)	2 (15.4)		99 (92.5)	8 (7.5)	0.5
ALP		8 (80)	2 (20)		87 (84.5)	16 (15.5)	1.0
GGT		8 (80)	2 (20)		98(91.6)	9 (8.4)	1.0
ALB	5 (38.5)	8(61.5)		9 (8.4)	98 (91.6)		0.063
PROT	0 (0)	13 (100)		1 (0.9)	106 (99.1)		1.0
TBIL		11 (84.6)	2 (15.4)		99(92.5)	8 (7.5)	0.5
DBIL		4 (100)	0 (0)		70 (65.4)	37(34.6)	1.0

## DISCUSSION

The overall prevalence of abnormal liver function tests in the study population was 57%. The most common abnormality was elevated direct bilirubin in 34.6% of participants, followed by elevated ALP in 15%. This study showed a low prevalence of ALT and AST abnormality at 4.7% and 7.5% respectively. Significant associated factors for elevated direct bilirubin included disease activity and duration of disease. Elevated direct bilirubin was more common in patients with a low disease activity and longer duration of disease. Duration of disease was a significant associated factor for both elevated ALP and direct bilirubin. Direct bilirubin and ALP may be elevated in case of liver disease and suggest intrahepatic biliary obstruction due to cholestatic liver disease.

Previous studies have noted liver dysfunction in RA at variable levels. Lefkovitz *et al* found a depressed albumin level as the most common LFT abnormality, which was in 37% of patients. Fewer patients had elevated ALP (15%) and bilirubin levels (0%). This study had a similar prevalence of ALP abnormality but higher bilirubin dysfunction. Notably, these were patients who had not yet been started on DMARDs and the difference in our population may well be an effect of treatment(39). Webb *et al* found that 18% of RA patients had elevated ALP, 1.9% of patients had elevated ALT and 0.5% of patients had elevated AST(23). In a review of RA patients on treatment with methotrexate, Amital *et al* found that a total of 45% of the tested patients had at least one abnormal result, most commonly ALP and albumin(100). This study is similar to this, and shows an overall prevalence of abnormal LFTs at 57%, with the common abnormal LFTs being direct bilirubin and ALP. A study done among RA patients on a combination of methotrexate and leflunomide by Curtis *et al* demonstrated LFT abnormalities in 33% of RA patients(42). These studies, done among patients who were on DMARDs have revealed variable prevalence of LFT abnormalities that have been attributed to the drug regimen, citing especially high dosage of drugs such as methotrexate, lack of folate supplementation and combination of drugs such as methotrexate and leflunomide.

In addition, this study reveals an elevation of direct bilirubin, which may not be fully explained by the effect of RA on the liver. An isolated elevation of direct bilirubin may be difficult to infer much from, though many recent studies have been evaluating elevated bilirubin as a protective factor in many inflammatory disease such as RA, SLE, stroke,

atherosclerosis and vasculitis (101). This could explain why elevated direct bilirubin was associated with low disease activity, as evidenced by this study. However, in combination with elevation of ALP, it may mean a cholestatic liver disease which may be due to the effect of RA.

The lower prevalence of abnormal ALP in this study population of 15% in comparison to the systematic review by Salliot *et al*, which had a prevalence of 20% (102), may be explained by use of folate supplementation, which all the study participants on methotrexate were on as well as the small number of patients on multiple DMARD combination. A total of 60% of patients were on methotrexate and 13.7% of patients were on both methotrexate and leflunomide. Methotrexate was the most commonly used DMARD in the study population, with 60% of study participants on it, and this corresponds to earlier studies done in this population (7). Folate was supplemented in all of the study participants on MTX and this has been proven to reduce adverse events of MTX, including gastrointestinal effects and liver function test abnormalities (103). This may explain the low number of liver function test abnormalities among those taking methotrexate. Kendall *et al* found the pathogenesis of abnormal LFT to be obscure and was unable to ascribe this to hepatotoxic drugs, alcohol or hepatitis(46).

This study shows no correlation of abnormal ALP with disease activity. There was however a protective effect of using DMARDs, especially prednisone and HCQS. Studies by Kendall *et al* and Cockel *et al* demonstrated that the abnormal LFTs, mostly ALP elevation, subsided with disease remission as well as steroid use in patients with RA(24,46). Lefkovitz *et al* also demonstrated a definite relationship between abnormal albumin and high disease activity(39).

The disease activity of this study's population which is high despite being on treatment could explain this difference. It was found that 97% of study participants have active disease. The population is also totally different from Lefkovits *et al*. This study was however unable to determine factors such as adherence of patients to medication prescribed, which may influence both the prevalence of abnormal LFTs as well as the disease activity. This study population is a group that is poorly controlled, with most patients having moderate to severe disease. This is higher than in the study by Owino *et al* in 2007(7), who found that at least 88% of RA patients had active disease. High disease activity among the RA patients may not be surprising, given that patients who have been on DMARDs for a long duration will have

poor response with time. The ERAN cohort in the UK similarly noted high disease activity using DAS28 in patients, despite being on DMARDs(104).

With longer disease duration, RA patients were almost three times more likely to have elevated direct bilirubin; OR 2.79 (1.23, 6.25), with a p value of 0.014. The effect of duration of disease could represent a cumulative effect of either the disease or the drugs on abnormal liver function. Though not significantly associated, being female and having a high education level seemed to be protective against development of abnormal bilirubin values. Drugs such as HCQS, especially at high doses were also protective against abnormal bilirubin results.

Notably, elevated ALP was significantly associated with BMI with an adjusted OR of 0.205 (0.074, 0.57), p value 0.002 as well as duration of disease, with an adjusted OR 1.14 (1.013, 1.29), p value 0.031. Kent *et al* showed an effect of obesity on liver function tests in RA patients using methotrexate(54). Other independent predictors of elevated ALP included occupation and use of oral contraceptives. Having an occupation appeared to be protective for elevated ALP, OR 0.41 (0.16, 1.01) with a p value of 0.05. However, use of OCP was up to 22 times predictive for elevated ALP OR 22.3 (1.72, 290.12), p value 0.018. Having been started on any DMARD and drugs such as HCQS, methotrexate and prednisone were noted to be protective against development of abnormal ALP.

Forty five percent of the study population was on treatment with low-dose steroids at the time of the study, with most having been on it for more than 1 year. A number of patients reported self- prescriptions with steroids since they felt it was more effective and a number reported poor compliance on the DMARD, citing better relief from the steroids or higher cost of the DMARD prescribed. This population also has high NSAID use, at 86%, which was not significantly associated with abnormal liver function tests, but could be a risk factor in their causation.

## **Study Limitations**

This was a cross-sectional study and participants were not followed up with serial LFT measurements. Liver function tests are non-specific and were only used to detect liver dysfunction and pattern of injury rather than point out to a specific liver disease. No invasive tests of liver function such as biopsy were performed and thus investigators were unable to



ascertain causes of liver dysfunction, especially those which may result from infection or autoimmune disease.

## **CONCLUSION**

This study reveals a high prevalence of abnormal LFTS, in 57% of RA patients. Such a high burden of liver dysfunction necessitates that LFTs should be a requirement in providing quality care to any patient with rheumatoid arthritis. Abnormal direct bilirubin was associated with a low disease activity and longer duration of disease. Duration of disease was a significant associated factor for both elevated ALP and direct bilirubin. Patients with rheumatoid arthritis who have had the disease for longer should be on the health provider's watch list and LFTs should be done often. None of the patients with abnormal LFTs had clinically evident liver disease, which therefore illustrates the importance of frequently monitoring LFTs among these patients. Moreover, most of the study population did not have previous liver function test results. This study therefore recommends regular monitoring of liver function tests in patients with rheumatoid arthritis, more so in those who have long-standing disease. Establishment of a prospective cohort would also be useful as well in determining the intervals at which monitoring for liver function should be done.

Further studies using more sensitive modalities to investigate liver dysfunction, such as liver elastography are warranted. Concurrent use of NSAIDs and DMARDs has been found to still pose a risk of 'silent' liver fibrosis, yet undetectable by liver biochemistry and thus more sensitive modalities would still be useful in this group of patients and may yield a higher prevalence of liver dysfunction.

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## **APPENDIX 1: INFORMED CONSENT**

### **Informed consent for Prevalence of abnormal liver function tests in Rheumatoid arthritis**

**Institution:** Department of Clinical Medicine and Therapeutics, College of Health Sciences  
University of Nairobi, P.O.BOX 30197-00400, Nairobi.

**Principal Investigator:** Dr. Agatha A. Olago-Rakuomi, P.O.BOX 30197-00400, Nairobi.

**Lead Supervisor:** Prof. Elly Ogutu

Department of Clinical Medicine and Therapeutics

### **Ethical Approval**

Kenyatta National Hospital /University of Nairobi Ethics and Research committee, P.O. BOX  
20723-00100, Nairobi. Tel 2726300/2716450 Ext 44102

### **INTRODUCTION**

I am undertaking a study investigating abnormal liver function tests in patients with Rheumatoid arthritis. This study is part of my University requirements but the results of the study will be used to offer recommendations which, if implemented, may lead to improved management and quality of life of patients with Rheumatoid arthritis.

This form is to give you the information you need before deciding if you want to participate in this study. As you read this form you may ask any questions on what you do not understand.

### **Purpose of the study**

I am carrying out a study in patients with Rheumatoid arthritis, to see how many have abnormal liver function tests and how this is associated with the disease and drugs taken.

### **Procedures to be followed in the study**

Once you agree to participate in the study, you will sign a consent form. You will then be asked questions regarding your condition as outlined in the study questionnaire. You will also undergo a physical examination after which we will draw a blood sample of about 5mls, or 1 teaspoon of blood.

### **Risks and costs incurred**

You may feel slight pain/ discomfort when the blood sample is drawn. There may be slight swelling at the site of the needle prick, but this will disappear on its own after a few days. The amount of blood that will be drawn will not affect your health.

### **Your rights as a participant**

Your participation in this research is voluntary and in the event that you refuse to participate in this study, your treatment will not be affected. If you choose to participate and not answer certain questions, you are free to do so. You are free to terminate the interview and withdraw from the study at any time. You are free to ask questions before signing the consent form.

### **Assurance of confidentiality**

All your responses as well as your results will remain confidential. Your individual responses will be stored in a locked place under my control and will only be seen by my statistician and I.

### **Benefits to you as a participant**

Your participation in the study and the laboratory test bear no cost to you but the findings will be used for your individual benefit. Information obtained will improve knowledge to health care givers at Kenyatta National hospital.

### **Contacts**

In case you need to contact me, my academic department or the Kenyatta national hospital / University of Nairobi Ethics and Research Committee concerning this study, please feel free to use the contacts provided above.

I request you to sign the consent form attached.

**Consent form**

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant:.....

Signature / Left thumbprint of subject:.....

Date:.....

**INVESTIGATOR’S STATEMENT:**

I, the Principal Investigator, have fully informed the research participant on the purpose and implication of this study.

Signed: .....

Date: .....

## **APPENDIX 2: FOMU YA MAELEZO YA UTAFITI**

### **Fomu ya maelezo ya utafiti wa kiwango cha maradhi ya ini katika wagonjwa wenye maumivu ya viungo**

**Taasisi:** Idhaa ya matibabu ya watu wazima, Chuo cha sayansi ya afya, Chuo kikuu cha Nairobi S.L.P. 30197-00400, Nairobi.

**Mtafiti mkuu:** Dkt. Agatha A. Olago-Rakuomi, S.L.P. 30197-00400, Nairobi.

Idhaa ya matibabu ya watu wazima

**Msimamizi mkuu:** Prof. Elly Ogutu, Idhaa ya matibabu ya watu wazima

### **Ridhaa:**

Kenyatta National Hospital /University of Nairobi Ethics and Research committee, S.L.P. 20723-00100, Nairobi. Tel 2726300/2716450 Ext 44102

### **Utangulizi**

Ninataraji kufanya uchunguzi kuhusu kiwango cha maradhi ya ini katika wagonjwa wenye maumivu ya viungo na ningependa uhusike. Utafiti huu unahitajika kama sehemu ya masomo yangu lakini matokeo yatakayopatikana yatumiwa kutoa maelezo, ambayo ikiwa itatumika italeti manufaa katika matibabu na hali ya maisha ya wagonjwa wa maumivu ya viungo.

Fomu hii ni ya maelezo yote utakayohitaji ukiamua kama utajiunga na utafiti huu. Unapoisoma na baada ya kusoma fomu hii, uko huru kuuliza maswali yoyote kama kuna sehemu hujaelewa vyema.

### **Je, utafiti huu unalenga kutambua nini?**

Ninafanya utafiti huu ili kukagua walio na maradhi ya ini katika wagonjwa wanaougua maumivu ya viungo, na vile ambavyo shida hii inalingana na ugonjwa wao na madawa wanayotumia.

### **Utaratibu wa utafiti:**

Mara utakapokubali kuhusika kwenye utafiti huu, utatia sahihi katika fomu ya ridhaa na matakwa ya utafiti. Itabidi ujibu maswali ya kibinafsi utakayoulizwa kisha utachunguzwa kimwili. Tutahitaji kuondoa mililita tano au kijiko moja ndogo ya damu.

### **Hatari na gharama inayohusika**

Unaweza hisi uchungu kidogo damu inapoondolewa. Mahali unapodungwa panaweza fura kidogo, lakini itaisha yenyewe baada ya siku chache. Damu itakayoondolewa ni kidogo na haitakudhuru.

### **Haki zako**

Kujiunga na utafiti huu ni kwa hiari yako. Hutabaguliwa kimatibabu ukikataa kujiunga na utafiti huu. Ukijiunga na utafiti huu na ushindwe kujibu mojawamo au maswali mengine tutakayouliza, ni sawa. Una uhuru wa kutoka kwenye mahojiano na kujitoa kwa utafiti huu wakati wowote. Una uhuru wa kuuliza maswali yoyote uliyo nayo kabla ya kutia sahihi fomu ya makubaliano. Maelezo yako yote yatawekwa pahali pa siri. Ni mtafiti mkuu na mwanatakwimu wake pekee ambao wataangalia maelezo yako.

### **Manufaa ya utafiti huu**

Hakuna pesa utahitajika kulipa kwa kujihusika kwa utafiti huu. Matokeo ya vipimo vya ini vitakufaidi kibinafsi. Matokeo ya utafiti yatasaidia wauguzi katika hospitali ya Kenyatta.

## **Cheti cha ridhaa**

Nimesoma, au nimesomewa maelezo yaliyopewa. Nimepata fursa ya kuuliza maswali kuhusu utafiti na maswali yote niliyouliza yamejibiwa vyema. Ninakubali kuhusika katika utafiti huu.

Jina la mhusika:.....

Sahihi/Alama ya kidole gumba cha kushoto :.....

Tarehe:.....

### **KAULI YA MTAFITI:**

Miye, mtafiti mkuu, nimemweleza mhusika vilivyo kuhusu utafiti huu.

Sahihi: .....

Tarehe:.....

## APPENDIX 3: DATA CAPTURE FORM

### Demographics

Study No: .....

Date:

Hospital No: .....

Age: .....

Sex: .....

Telephone contact: .....

Physical Address: .....

Occupation: .....

Highest level of education: None..... Primary..... Secondary.....

Tertiary.....

Marital Status: Single ..... Married..... Widowed.....

### Disease history

History of RA: When was the diagnosis of RA made?

< 1 year ago .... 1-5 years .... > 5 years.....

Rheumatoid factor available? Positive..... Negative.....

Anti CCP available? Positive..... Negative.....

Has any medication been started? Yes .... No ....

Hepatitis B Vaccination? Yes..... No.....



Previous LFT result (within last 6 months) available? Yes..... No.....

Liver function test	Reference range	Actual Result
ALT	5-40 IU/L	
AST	3-35IU/L	
ALP	40-150IU/L	
GGT	0-50IU/L	
Albumin	37-60g/L	
Protein	60-80g/L	
Total Bilirubin	1.7-20.6µmol/	
Direct Bilirubin	0-8.6µmol/L	
Hep B		
Hep C		

Which medication and dosage have you been using?

a) NSAIDs: Yes .... No ....

If yes, for how long? <3 months .... 3 months – 1 year .... >1 year.....

Dosage.....

b) DMARDs

i) MTX: Yes .... No ....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year ....

Dose.....

Supplementation with Folic acid? Yes..... No.....

ii) HCQS: Yes .... No ....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year ....

Dose.....

iii) Steroids: Yes .... No ....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year .....

Drug..... Dose.....

iv) Leflunomide: Yes .... No ....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year ....

Dose.....

v) Sulfasalazine: Yes .... No ....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year ....

Dose.....

vi) Biologic agents: Yes .... No ....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year ....

Dose.....

Any other diseases currently being managed:

HIV

DM

Hep B

Hep C

Thyroid disease

Other medications currently being taken;

Statins: Yes .... No ....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year ....

Antiepileptic drugs Yes .... No ....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year ....

Oral contraceptives Yes .... No ....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year ....

Alcohol intake: Never..... Yes..... Not currently.....

If yes, how many days have you drunk alcohol in the last one month?

1-5 days..... 6-10 days..... >10 days.....

Type of alcohol taken..... Amount/day.....

How many years have you taken alcohol?

If you stopped taking alcohol, how long ago did you stop?

Use of herbal medication: Yes..... No.....

If yes, for how long? < 3months .... 3 months – 1 year .... >1 year .....

Smoking status (pack years): Current..... (...) Previous smoker..... (.....)

Never smoked.....

### **Examination**

BMI: .....

Jaundice: Yes..... No.....

Liver size (cm) .....

Spleen (palpable): Yes..... No.....

## Laboratory Results

Liver function test	Reference range	Actual Result
ALT	5-40 IU/L	
AST	3-35IU/L	
ALP	40-150IU/L	
GGT	0-50IU/L	
Albumin	37-60g/L	
Protein	60-80g/L	
Total Bilirubin	1.7-20.6 $\mu$ mol/	
Direct Bilirubin	0-8.6 $\mu$ mol/L	

**APPENDIX 4: ACR/EULAR CRITERIA FOR DIAGNOSIS OF RHEUMATOID ARTHRITIS**

<b>JOINT DISTRIBUTION (0-5)</b>	
1 large joint	0
2-10 large joints	1
1-3 small joints (large joints not counted)	2
4-10 small joints (large joints not counted)	3
>10 joints (at least one small joint)	5
<b>Negative RF <u>AND</u> negative ACPA</b>	
Low positive RF <u>OR</u> low positive ACPA	2
High positive RF <u>OR</u> high positive ACPA	3
<b>SYMPTOM DURATION (0-1)</b>	
<6 weeks	0
≥6 weeks	1
<b>ACUTE PHASE REACTANTS (0-1)</b>	
Normal CRP <u>AND</u> normal ESR	0
Abnormal CRP <u>OR</u> abnormal ESR	1

A score of more than 6 is definite RA.

If the score is less than 6, a patient might fulfill the criteria prospectively over time (cumulatively) or retrospectively if data on all four domains have been adequately recorded in the past.

## APPENDIX 5: DISEASE ACTIVITY SCORE IN 28 JOINTS (DAS28)

### Disease Activity Score in 28 Joints (DAS28)

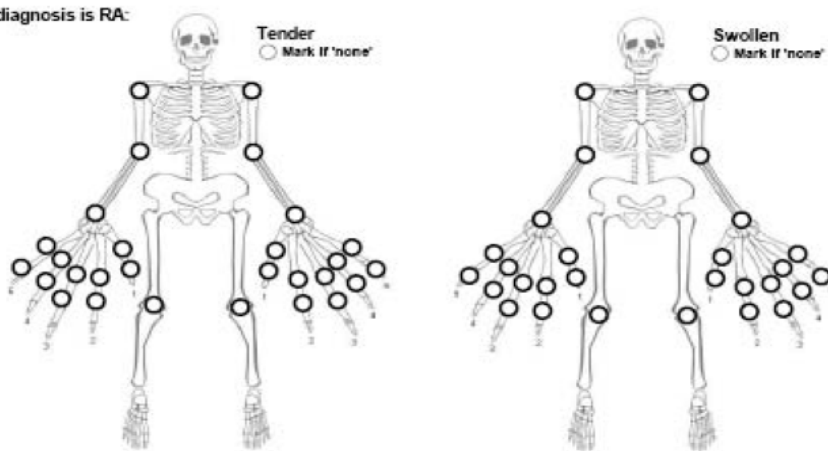
#### Patient global assessment

Considering all the ways in which illness and health may affect you at this time, please indicate below how you are doing:

VERY WELL |-----| VERY POORLY

FOR PROVIDER USE ONLY

If diagnosis is RA:



VAS (0-100)

28TJC

28SJC

ESR

DAS28

$$\text{DAS28} = 0.56 \cdot \sqrt{28\text{TJC}} + 0.28 \cdot \sqrt{28\text{SJC}} + 0.70 \cdot \ln(\text{ESR}/\text{CRP}) + 0.014 \cdot \text{VAS}$$

How to calculate a DAS28 score:

1. Ask the patient to make a vertical mark on a 100 mm Visual Analog Scale (VAS) corresponding to their general health or global disease activity. Using a ruler, measure from the left-hand side in mm. Note: DAS28 calculations may be performed without a VAS measurement.
2. Perform a swollen and tender joint examination on your patient. Add all of the swollen and tender joints and record the totals in the appropriate boxes.
3. Erythrocyte Sedimentation Rate (ESR) should be measured (in mm/hour). Note: C-reactive protein (CRP) levels may be used as a substitute for an ESR.
4. Plug the appropriate values into the formula (many online calculators are available including <http://www.das-score.nl/www.das-score.nl/dascalators.html>).
5. If using CRP instead of ESR or calculating a score from only 3 variables please see <http://www.reuma-nijmegen.nl/www.das-score.nl/> for the appropriate formula.

Interpretation:

- The DAS28 provides you with a number on a scale from 0 to 10 indicating current RA disease activity.
- Remission:  $\text{DAS28} \leq 2.6$
- Low Disease activity:  $2.6 < \text{DAS28} \leq 3.2$
- Moderate Disease Activity:  $3.2 < \text{DAS28} \leq 5.1$
- High Disease Activity:  $\text{DAS28} > 5.1$

Adapted from: DAS-Score.nl. Available at <http://www.das-score.nl/www.das-score.nl/index.html>. Accessed April 15, 2010.

## APPENDIX 6: ETHICAL APPROVAL



UNIVERSITY OF NAIROBI  
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Ref: KNH-ERC/A/38

1<sup>st</sup> February, 2016

Dr. Agatha Adhiambo Olago- Rakuomi  
Reg. No. H58/63986/2013  
Dept. of Clinical Medicine and Therapeutics  
School of Medicine  
College of Health Sciences  
University of Nairobi

Dear Dr. Olago- Rakuomi,

### **Revised research proposal: Prevalence of Abnormal Liver Function Tests in Rheumatoid Arthritis (P771/12/2015)**

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and **approved** your above proposal. The approval period is from 1<sup>st</sup> February 2016 – 31<sup>st</sup> January 2017.

This approval is subject to compliance with the following requirements:

- Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
- All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
- Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
- Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
- Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
- Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
- Submission of an *executive summary* report within 90 days upon completion of the study.  
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website <http://www.erc.uonbi.ac.ke>



Yours sincerely,

**PROF. M.L. CHINDIA**  
**SECRETARY, KNH-UoN ERC**

- c.c. The Principal, College of Health Sciences, UoN  
The Deputy Director, CS, KNH  
The Chair, KNH-UoN ERC  
The Assistant Director, Health Information, KNH  
The Dean, School of Medicine, UoN  
The Chair, Dept. of Clinical Medicine and Therapeutics, UoN  
Supervisors: Prof. Elly Otieno Ogutu, Prof. George Omondi Oyoo, Dr Edna Wairimu Kamau, Dr. Eugene Genga  
Kalman