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Original Article

Impact of chronic low dose methotrexate treatment in rheumatoid arthritis on biochemical parameters in a population of eastern Algeria: A cross-sectional study

Impact du traitement chronique par méthotrexate à faible dose au cours de la polyarthrite rhumatoïde sur les paramètres biochimiques dans une population de l'Est algérien : Étude transversale

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ABSTRACT

Background: Lack of data describing biochemical parameters' status in Algerian population during rheumatoid arthritis with long-term low dose methotrexate treatment. **Objectives:** Evaluation of biochemical markers' concentration and prevalence of values outside standards. **Patients and Methods:** A cross-sectional study was conducted from October 2014 to March 2016 at Batna University Hospital, Algeria. Biochemical parameters' assays were performed on Dimension RxL/Immulite 2000XPi and methotrexatemia determination on Unicel DxC600. Statistical tests were run on SPSS Statistics with a p value <0.05. **Results:** A total of 91 patients were recruited with a M/F sex ratio of 0.30 and a median age of 49 years (36-59). All patients received a median methotrexate dosage of 15 mg/week with folinic acid supplementation in 71 patients. No significant differences in biochemical parameters concentration or disturbed values prevalence were noted between patients with at least one risk factor and those with none. Creatinine clearance was lower in patients with a methotrexate cumulative dose ≥ 1.5 g (p = 0.036). Disturbed creatinine clearance values (<80 mL/min) were noted only in female patients with a prevalence of 13.18%. Hyperhomocysteinemia (> 15 µmol/L) was found in 27.47% of study population with a higher prevalence of 47.82% during vitamin B_{12} deficiency. **Conclusion:** Despite a good overall tolerance, it is recommended to monitor creatinine clearance especially in female patients and from a methotrexate cumulative dose exceeding 1.5 g. To avoid any atherosclerosis risk, periodic cardiovascular function monitoring, a blood count formula and a lipid profile should also be realized, particularly during vitamin B_{12} deficiency.

KEYWORDS: Methotrexate, rheumatoid arthritis, biochemistry, eastern Algeria.

RESUME

Introduction : Manque de données décrivant le statut des paramètres biochimiques de la population algérienne au cours de la polyarthrite rhumatoïde lors d'un traitement chronique à base de méthotrexate à faible dose. **Objectifs :** Évaluation de la concentration des marqueurs biochimiques et des valeurs hors normes. **Patients et Méthode :** Étude transversale, conduite d'octobre 2014 à mars 2016 au CHU Batna, Algérie. Les analyses biochimiques ont été effectuées sur Dimension RxL/Immulite 2000XPi et la méthotrexatémie dosée sur Unicel DxC600. Les tests statistiques ont été réalisés sur SPSS Statistics ($p < 0,05$). **Résultats :** 91 patients ont été recrutés avec un sex-ratio H/F de 0,30 et un âge médian de 49 ans (36-59). La dose médiane de méthotrexate était de 15 mg/semaine, avec supplémentation en acide folinique chez 71 patients. Aucune différence significative n'a été notée pour la concentration des paramètres biochimiques ou la prévalence des valeurs perturbées, entre les patients avec au moins un facteur de risque et ceux n'en présentant aucun. La clairance de la créatinine était plus faible chez les patients avec une dose cumulée de méthotrexate ≥ 1,5 g ($p = 0,036$). Des valeurs perturbées (<80 mL/min) n'ont été notées que chez les patients de sexe féminin avec une prévalence de 13,18%. Une hyperhomocystéinémie (> 15µmol/L) a été notée chez 27,47% des patients avec une prévalence plus élevée, de 47,82%, en cas de carence en vitamine B12. **Conclusion :** Bien qu'une bonne tolérance ait été notée, il est recommandé de surveiller la clairance de la créatinine surtout chez les patients de sexe féminin, à partir d'une dose cumulée de 1,5 g de méthotrexate. Pour éviter tout risque d'athérosclérose, une surveillance périodique de la fonction cardiovasculaire, une formule de numération sanguine ainsi qu'un bilan lipidique doivent également être réalisée, notamment en cas de carence en vitamine B12.

MOTS CLES: Méthotrexate, polyarthrite rhumatoïde, biochimie, Est algérien.

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Introduction

Rheumatoid arthritis is a chronic disease whose treatment involves drugs implying different mechanisms of action.

Methotrexate (MTX) is an antimetabolite used at high dose as a folic acid antagonist and an inhibitor of dihydrofolate reductase in malignant diseases treatment especially leukemias. It's also widely used at low doses as a disease-modifying antirheumatic drug (DMARD). The Food and Drug Administration approved its use in 1972 in psoriasis treatment and in 1988 for rheumatoid arthritis (RA), becoming one of the most prescribed drugs in dermatology and rheumatology **[1-3]**.

Chronic low-dose treatments have also been implicated in toxicities occurrence including liver and kidney side effects that may be responsible for treatment discontinuation **[4-6]**. In order to reduce these adverse reactions, folinic acid supplementation is recommended and represents the main antidote against MTX toxicities **[7]**.

Higher cardiovascular mortality is also reported in RA patients **[8]**, an incidence that could be aggravated during MTX therapy, the latter may be responsible for homocysteine increases **[9]**, a sulphur-containing amino acid potentially involved, as an independent risk factor, in endothelium alteration and cardiovascular diseases. Increases that have also been associated with vitamin B_{12} and folic acid deficiencies **[7, 9]**.

A multicenter study examining RA patients' characteristics in Algeria revealed that 89.7% of them were taking DMARDs, where MTX was the most commonly used drug (72.2%) **[10]**. However, to the best of our knowledge, no work has been published describing biochemical parameters' state during a long-term low dose MTX treatment.

In order to provide more information on the subject, it was decided to assess the impact of MTX chronic treatments on the biochemical parameters' plasma values. The evaluation of the MTX chronic therapy impact focused on renal and hepatic biochemical markers monitoring, as well as the examination of homocysteine, vitamin B_{12} and folic acid levels.

Patients and Methods

Patients and study design

A cross-sectional study was conducted in compliance with the STROBE guideline, over a period of 18 months (from October 2014 to March 2016), after approval from the Scientific Council of Medicine Faculty, Batna 2 University (minutes' reference number: N°35/CSF/FM/2013) and according to the Helsinki Declaration. All recruited patients received low dose MTX and were treated for RA. A sample size of at least 58 patients was fixed in accordance to the study design, with a precision of 0.01, knowing that RA prevalence in the Algerian population was estimated at 0.15% **[11]**. Enrolment was conducted by liberal and hospital rheumatologists from Batna, Algeria. Samples' collection and analyses were carried out at Benflis Touhami, Batna and Mustapha, Algiers, University Hospitals. Patients with lack of data were excluded from the study.

For each patient, an information sheet was drawn up with the following information: age, sex, body surface area (BSA), body mass index (BMI), other pathologies than RA, smoking/alcoholism notion, occupation and other treatments received. Informed consent was obtained and archived for all included participants.

Risk factors that could impact the liver and kidney function were also noted: obesity (BMI> 30), diabetes, liver, kidney or heart disease, positive viral serology (hepatitis B and C, HIV), Wilson's disease, Sjörgen's syndrome, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, statins, vitamin A, homeopathic treatment, anti-tuberculosis drugs and PUVA therapy **[12, 13]**. A comparison of biochemical parameters' concentrations and the prevalence of disturbed values were realized between patients with at least one risk factor and those with none.

Knowing that a liver biopsy is also recommended beyond a MTX cumulative dose of 1.5 g **[1, 2, 5]**, a comparison was carried out between patients receiving dosage above and below this limit.

Samples and analysis methods

Venous blood's samples were collected after 12 h of fasting, in dry and heparinized tubes. Hemolyzed or icteric samples were rejected. Centrifugation was carried out immediately during 10 minutes at 3000 rpm. In case of analysis postponement, samples were stored at -20 ° C.

MTX was assayed by enzyme immunoassay (Siemens Syva EMIT) on UniCel DxC 600 (Beckman Coulter, Inc., Brea, CA 92821 USA) with a limit of quantification of 0.05 µmol/L. Biochemical monitoring included the following parameters: urea, creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and creatine phosphokinase (CK). Analyses were performed on Dimension RxL (Siemens Healthcare Diagnostics Inc., Newark, DE 19714 USA). Creatinine Clearance (CrCl) was estimated by the Cockcroft and Gault (CG) formula. Total plasma homocysteine, folic acid and vitamin B_{12} were assayed on Immulite 2000 XPi (Siemens Healthcare Diagnostics Inc., USA).

Statistical analysis

Normal distribution evaluation was carried out by one-sample Kolmogorov-Smirnov test. Data were not normally distributed and thus presented as median and interquartile range. Comparison was carried out by Mann Whitney U and Chi-squared (χ^2) tests. Statistical analyses were performed on IBM SPSS Statistics version 22. Statistical significance was set at $p < 0.05$.

Results

A total of 91 RA patients were recruited with demographic, clinical and biochemical characteristics as presented in Table 1.
A male/female sex ratio of 0.30 was noted with 64.83% of the study population without occupation. All patients received MTX with a median dosage of 15 mg/week. Folinic acid supplementation was prescribed in 71 patients with a dosage range varying from 5 to 15 mg/week. Concerning other medications, 27 subjects were taking a combination of two or more DMARDs (23 received hydroxychloroquine, 4 sulfasalazine and 2 leflunomide), 58 were taking glucocorticoids, 37 NSAIDs, 28 calcium/vitamin D supplementation and only one was taking biologics (tocilizumab). All patients presented a good MTX elimination with serum concentrations < 0.05 µmol/L.

One risk factor at least was found in 56 patients. Only three were smokers and none consumed alcohol. Prevalence of biochemical parameters' values outside standards has proven to be low (Table 2) with no significant differences in biochemical parameters' concentration or the prevalence of disturbed values between patients with at least one risk factor and those with none.

Frequencies were however relatively higher for parameters related to liver function (AST, ALT, GGT,

ALP) and CrCl. It is noteworthy that all CrCls outside reference ranges were found in female patients.
In patients receiving dosage above and below a MTX cumulative dose of 1.5 g, only creatinine and CrCl showed significant differences, with respectively 71 µmol/L (62-80 µmol/L) vs. 62 µmol/L (53-71 µmol/L) (p=0.037) and 97.65 mL/min (90.28-115.10 mL/min) vs. 113.33 mL/min (94.23-143.33 mL/min) (p=0.036).

Table 1: Patients' characteristics

Parameters	Median	Interquartile Range
Age (years)	49	36-59
Sex ratio (male/female)	21/70	-
BSA (m^2)	1.76	1.65-1.87
BMI (Kg/m^2)	27.64	24.09-29.68
MTX dosage (mg/week)	15	10-15
MTX treatment duration (months)	19	9-34
MTX cumulative dose (g)	0.92	0.48-1.75
Folinic acid dosage (mg/week)	10	5-10
Urea (mmol/L)	4.6	3.8-5.5
Creatinine (µmol /L)	62	53-71
Uric acid (µmol/L)	250	208-291
CrCl (mL/min)	109.20	91.52-129.74
AST (U/L)	25	20-32
ALT (U/L)	21	13-29
ALP (U/L)	91	76-111
GGT (U/L)	22	14-29
CK (U/L)	77	46-108
Homocysteine (µmol/L)	11.35	9.61-15.50
Vitamin B_{12} (pmol/L)	181	139-284
Folic acid (nmol/L)	27.40	15.43-47.35

Out of all patients, 25 presented hyperhomocysteinemia (> 15 µmol/L) and only two had moderate increases (30 - 100 µmol/L) with concentrations of 41.40 and 39.40 µmol/L.

Vitamin B_{12} deficiency was found in 23 patients that showed a significantly higher concentration of homocysteine and a more important hyperhomocysteinemia prevalence compared to others, with 14.50 µmol/L (10.10-21.90 µmol/L) vs. 11.10 µmol/L (9.60-13.72 µmol/L) (p=0.046) and 47.83% (95% CI 25.74-69.91%) vs. 20.59% (95% CI 10.73-30.45%) (p=0.014 with Relative Risk of 2.320), respectively.

Table 2: Prevalence of biochemical parameters' values outside standards.

Parameters	Standards	Prevalence of values outside standards
Urea (mmol/L)	< 8.3	4.39%
Creatinine (µmol /L)	< 106	1.09%
Uric acid (µmol/L)	< 417	4.39%
CrCl (mL/min)	> 80	13.18%
AST (U/L)	< 40	12.08%
ALT (U/L)	< 40	6.59%
ALP (U/L)	< 130	13.18%
GGT (U/L)	< 61	7.69%
CK (U/L)	< 171	6.59%
Homocysteine (µmol/L)	> 15	27.47%
Vitamin B_{12} (pmol/L)	< 141	25.27%
Folic acid (nmol/L)	> 7.0	3.29%

Discussion

As a gold standard, MTX is widely used during RA therapy in Algeria. Special attention from physicians focuses on adverse effects treatment, by clinical monitoring in addition to biochemical parameters analysis that helps predicting toxicities occurrence. However, no study to date indicates the prevalence of their disturbance in Algerian population, a situation that led to the realization of the present study enabling a more appropriate approach and a better care of patients.
Among the monitored biochemical parameters, liver enzymes are especially controlled after a long MTX treatment period, although recent study on other populations show low correlation between cumulative dose and fibrosis **[4]**. This seems to be the case during the present work where no significant transaminases' values elevation is noted beyond a MTX cumulative dose of 1.5 g (p = 0,879 for AST and p = 0,917 for ALT). A result that can be explained by recruited patients, affected by RA, that seem to have a better MTX tolerance compared to psoriatic ones **[12, 14]**. Indeed, although transaminases concentration elevation correlates little with histological changes and fibrosis risk **[15-17]**, a study showed that prevalence of elevated liver enzymes concentration during MTX treatments in psoriasis cases was higher comparatively to RA (14.5% vs. 7.5%) **[13]**. These results are close to those found in the present study where occurrences of AST and ALT values outside reference ranges are of 12.08% and 6.59% respectively. A genetic predisposition to MTX hepatotoxicity in psoriatic patients is also suggested

[13]. Nevertheless, biopsy remains the only way to confirm fibrosis and it's still recommended in cases of persistent transaminases' values elevation, despite possible complication inherent to its practice and its high cost **[4, 12, 15]**.

Renal function plays as well a major role in MTX-based treatment where decreases in CrCl may lead to dosage adjustment with a 50% dose reduction in case of CrCl < 45 mL/min **[6, 18]**. According to the study data, renal function seems to be significatively affected by MTX cumulative dose ($p = 0.037$ for creatinine and $p = 0.036$ for CrCl), especially in female patients. A significatively higher CrCl disturbance prevalence is also noted, compared to creatinine (13.18% vs 1.09% respectively, $p < 0.001$), privileging CrCl in kidney monitoring, for which it is recommended an estimation by the CG formula **[19]**.

A higher cardiovascular mortality reported in RA patients could also be aggravated by the MTX therapy, involved in homocysteine concentration elevation. Our study reveals a median plasma homocysteine concentration that is close to other papers' results where patients were taking low dose MTX with folinic and folic acid supplementation (11.35 vs. 14.30 - 10.90 µmol/L respectively) **[20, 21]**. It is noteworthy that hyperhomocysteinemia prevalence is significantly higher in cases of vitamin B_{12} deficiency which implies more careful cardiovascular function monitoring in this group knowing that low vitamin B_{12} concentration is also considered as an atherosclerosis risk factor partially independent of homocysteine **[22]**.

Regarding risk factors, the present study shows no significant differences in biochemical parameters' concentration or disturbed values prevalence between patients with and without risk factor. This good tolerance can be explained by recruited patients' profile, treated for RA and where most of them received concomitant folinic acid supplementation. Population habits may also explain this low side effects prevalence, with relatively low proportion of obesity and alcohol consumption **[23, 24]** in addition to the fact that RA in Algerian population appears to be less aggressive compared to Western ones **[10]**.

Although the study design only gives a snapshot of biochemical parameters' values, the results allow us to gather essential information for future investigation. The study also provides clinicians with a set of data useful in their daily practice when using MTX and ensures a better patient follow-up.

Conclusion

On the basis of our results and despite a good overall tolerance, it is recommended for the Algerian population under low dose MTX therapy, to monitor CrCl especially in female patients and from a MTX cumulative dose exceeding 1.5 g. To avoid any atherosclerosis risk, periodic cardiovascular function monitoring should also be realized, particularly during vitamin B_{12} deficiency that may be accompanied by hyperhomocysteinemia where it is also recommended to perform a blood count formula, including reticulocyte level, as well as a lipid profile, in order to prevent any complications.

Conflicts of interest

Authors do not declare any conflict of interest.

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