

Assessment of Tumor Necrosis Factor Alpha, Homocysteine and Pro-Collagen type III Peptide as Markers in Patients with Moderate to Severe Psoriasis

Dr. Mohammed I. Hamza¹, Hasan N. AL_musawi², Anwar H. Mathboob³

¹ Assistant Professor / University of Al-Nahrain/College of Medicine/Department of Chemistry and Biochemistry

²Lecture/ University of Al-Nahrain /College of Medicine/Department of Medicine/Dermatology

³ Directorate of Wasit health / Iraq / Wasit governorate

Abstract: *Background:* psoriasis is chronic and recurrent autoimmune inflammatory disease cause morbidity and mortality. TNF α is a Pro-inflammatory cytokine and has a key role in the pathogenesis of psoriasis. Homocysteine level in psoriasis and cause hyperhomocystinaemia that play role in atherosclerosis and .Procollagen type III peptide is fibro genesis marker methotrexate is widely used in treatment of moderate to severe psoriasis and this lead to develop liver fibrosis, hence measurement of procollagen type III peptide may reduce liver biopsy. *Objective:* 1- measured the level of (TNF α), Homocystiene and procollagen type III in patient with moderate and sever psoriasis and compared the result with healthy control. 2- the level of (TNF α), Homocystiene and PIIP in patients before methotrexate and after 4week treatment. *Subjects, materials and methods:* This observational case control study involved thirtypatients with moderate tosevere psoriasis and thirty healthy control with matched gender and age with patient groups, used ELISA technique for measured of (TNF α and, PIIP). The Homocystiene is measured by used HPLC technique through de-deproteinization the serum and then mixed with POA reagent for analysis. *Results:* Mean serum Homocystiene was significantly higher in patient group than in control group, 106.42 \pm 54.68 versus 50.07 \pm 24.16 ($P<0.01$), and it underwent no significant change after treatment with methotraxate to a level of 99.11 \pm 6.26 ($P=0.435$). Mean serum Procollagen was significantly higher in patient group than in control group, 31.85 \pm 13.84 versus 19.42 \pm 2.11 ($P<0.001$), and it underwent significant reduction after treatment with methotraxate to a level of 25.60 \pm 8.34 ($P=0.002$). Mean serum TNF was significantly higher in patient group than in control group, 16.13 \pm 2.09 versus 10.25 \pm 1.04 ($P<0.001$), but it showed insignificant change after treatment with methotraxate to become 16.18 \pm 4.38 ($P=0.949$). Significant positive correlation between Homocystien before and after MTX treatment and Pro-collagen III before and after treatment with MTX in patient group, were obtained. *Conclusion:* Homocystein is raised in psoriasis and hyperhomocystinaemia predispose to atherosclerosis; Pro-collagen III peptide is elevated in psoriasis and not affected by short term treatment with MTX, the TNF α is raise in psoriasis and stay higher after MTX treatment. Methotrexate in low dose is safe in patients with moderate to severe psoriasis; however monitoring of renal and liver function tests and hematologic parameters is necessary.

Keywords: Pro-collagen, Homocystein, TNF α , Psoriasis

1. Introduction

Psoriasis is defined as a chronic and recurrent autoimmune inflammatory dermatosis, characterized by well demarcated, scaling, and red plaques with recurrent attacks happening during a patient's lifetime [1]. Wide variation in prevalence rate has been reported globally; the lowest rate of 0.09% was recorded in the United Republic of Tanzania [2], whereas the highest rate was reported in Norway, 11.4% [2]. The Tumor Necrosis Factor alpha (TNF α) is a pro-inflammatory cytokine which can be secreted by immunological cells and has a key role in the pathogenesis of psoriasis so, target therapies that are directed against TNF α receptors were shown to be effective in treating psoriasis [3]. Procollagentype III peptide has one of the most frequently studied fibrogenesismarker. Methotrexate is widely used in the treatment of moderate to severe psoriasis and this may lead to development of liver fibrosis in a minor fraction of patients; hence serial measurements of serum procollagen III peptide may reduce the need for liver biopsy to investigate for liver fibrosis [4];Hyperhomocystinaemia palys a role as a risk factor for development of atherosclerotic ischemic heart disease and CVA [5], elevated homocysteine levels in

patients with psoriatic rendering them more liable for development of vascular endothelial injury with subsequent atherosclerosis and its complications [6]

2. Patients, Materials and Methods

This observational case control study involved thirty patients (16 females and 14 males) with moderate to severe psoriasis who were visiting the outpatient clinic of Dermatology at Imamein Kadhimein Medical City during period from December 2015 through May 2016. Their age ranged from (20-60) years, and 30 age and gender matched apparently healthy subjects. All patients started methotrexate (MTX) treatment after a baseline liver function test, renal function test and hematological test. All patients and control subjects were subjected to the following investigation: serum TNF- α , serum pro-collage III peptide and serumhomocystein. Comparison of these variables was done between patients and control groups. Also before and 4 weeks after MTX treatment in patients group.

3. Statistical

Data were summarized, analyzed and presented using two software programs; these were Statistical Package for Social Sciences (SPSS version 20) and Microsoft Office Excel 2010. Scale variables were presented as mean \pm Standard Deviation (SD) whereas categorical variables were expressed as number and percentage, Wilcoxon test was used in cases of non-normal distribution; on the other hand independent samples t-test was used to compare mean between two groups when assumption of normal distribution was obtained whereas Mann Whitney U test was used in case of non-normal distribution. Association between categorical variables was assessed by using Chi-Square test. Spearman and Pearson correlation coefficient tests were used to study correlation among scale variables.

Correlation coefficient tests were used to study correlation among scale variables.

4. Results

Mean serum Homocystien was significantly higher in patient group than in control group, 106.42 ± 54.68 versus 50.07 ± 24.16 ($P < 0.01$), and underwent no significant change after treatment with methotraxate to a level of 99.11 ± 6.26 ($P = 0.435$), table (1) and figure (1)

Mean serum Procollagen was significantly higher in patient group than in control group, 31.85 ± 13.84 versus 19.42 ± 2.11 ($P < 0.001$), and it underwent significant reduction after treatment with methotraxate to a level of 25.60 ± 8.34 ($P = 0.002$), table (4) and figure (2).

Mean serum TNF- α was significantly higher in patient group than in control group, 16.13 ± 2.09 versus 10.25 ± 1.04 ($P < 0.001$), but it showed insignificant change after treatment with methotraxate to become 16.18 ± 4.38 ($P = 0.949$), table (1) and figure (3)

There is positive correlation between homocystiene and PIIP before and after four weeks, through Methtrexate treatment figure (4), (5)

Table 1: Mean Homocystien, Procollagen and TNF α in patients group and control group

| Parameter | | Mean \pm SD | Range | P1 | P2 |
|---|---------|--------------------|---------------|--------|--------|
| Homocystien (HCY) | Before | 106.42 ± 54.68 | 14.60 -178.41 | <0.001 | 0.435 |
| | After | 99.11 ± 6.26 | 86.00 -109.30 | | |
| | Control | 50.07 ± 24.16 | 26.98 -85.78 | | |
| Pro – collagen(PIIINP) | Before | 31.85 ± 13.84 | 15.60 -64.83 | 0.001 | <0.002 |
| | After | 25.60 ± 8.34 | 13.76 -40.33 | | |
| | Control | 19.42 ± 2.11 | 15.93 -22.59 | | |
| Tumor necrosis factor alpha(TNF- α) | Before | 16.13 ± 2.09 | 11.53 -19.10 | 0.001 | <0.949 |
| | After | 16.18 ± 4.38 | 10.78 -28.80 | | |
| | Control | 10.25 ± 1.04 | 8.31 -12.21 | | |

P1: patientsvs control. P2: patients before vs after, (MTX)

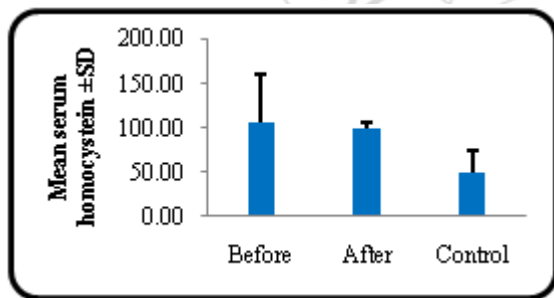


Figure 1: Mean serum homocystien in patients and control group

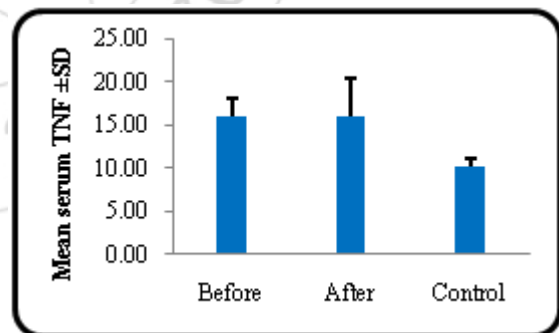


Figure 3: Mean serum TNF in patients and control group

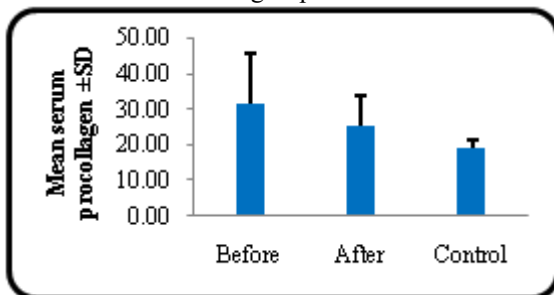


Figure 2: Mean serum procollagen in patients and control group

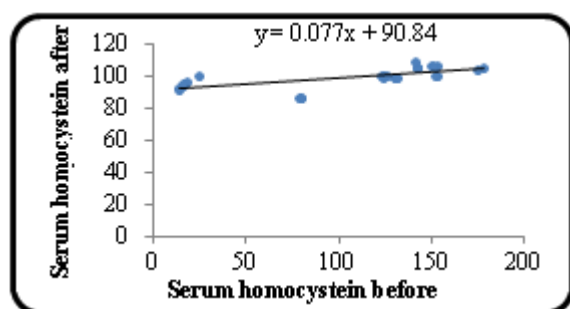


Figure 4: Correlation between serum homocystien before and after (MTX) treatment in patients group

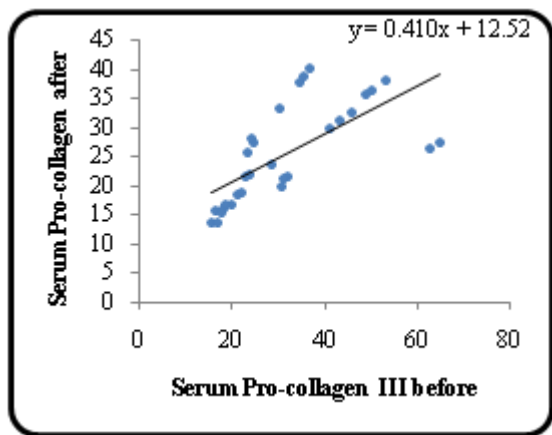


Figure 5: Correlation between serum pro-collagen III before and after (MTX) treatment in patients group

5. Discussion

The current study came up with an observation that serum homocysteine was significantly higher in patients with psoriasis than in control group prior to commencing methotrexate treatment. Several studies reported similar observation [7]. The proposed mechanism for the increase homocysteine in patients with psoriasis may be related to low serum folate that is caused by the associated inflammatory changes within intestinal mucosa making the absorption of dietary folate less than normal [8], nevertheless; amore rational explanation may be related to rapid skin turn over with subsequent utilization of folate leading to low serum folate [6]. Folate is essential for the action of the enzymes, acting as a co-enzyme, that are responsible for the metabolism of homocysteine, hence when folate is low homocysteine level will be elevated [9]. A high level of homocysteine increases susceptibility to endothelial injury, which leads to vascular inflammation. This in turn may lead to atherogenesis, which can result in ischemic injury [7]. The present study showed no significant alteration in serum homocysteine in patients with psoriasis following treatment with methotrexate in agreement with [10]. On the other hand the low dose methotrexate causes a rise in homocysteine level which was corrected by adding low dose folate supplementation [11]. The lack of significant change in homocysteine in the present study, following methotrexate treatment may be related to the fact that patients were on folate supplement in addition to low dose methotrexate.

The present study showed that serum pro-collagen III is significantly higher in psoriatic patients in comparison to healthy control group and that no further significant rise in its level to be attributed to one month low dose treatment with methotrexate. The proposed explanation for the significantly higher serum pro-collagen III in psoriatic patients, compared to control group, is that psoriasis is a risk for metabolic syndrome [12] and the development of non-alcoholic fatty liver with subsequent hepatitis and fibrosis [13]. On the other hand the current study failed to elicit a raised pro-collagen level following treatment with methotrexate. Most literatures that showed raised serum pro-collagen III, did so following serial measurement in patients with long term low dose methotrexate treatment [14], hence a single reading may be not sufficient to show a significant

rise in pro-collagen III which is a marker of liver fibrosis. The validity of using pro-collagen III in patients on low dose methotrexate treatment is not to diagnose liver fibrosis rather than to minimize the need for follow up liver biopsies number in patients on long term treatment with methotrexate [15]. It is likely that PIIINP in serum reflects the activity of fibrogenesis at the time of sampling rather than the degree of established fibrosis seen on biopsy [4]. PIIINP should therefore be measured serially to be a valid test, and we recommended measuring it every 3 months in patients on long-term MTX for psoriasis [4]. A multicentre audit confirmed the usefulness of PIIINP in psoriatic patients on MTX and showed that patients monitored using serial PIIINP measurement and selective liver biopsy were subjected to 7-fold fewer liver biopsies compared with those managed according to the guidelines of the American Academy of Dermatology without the evidence that important liver toxicity was missed [16]. Release of PIIINP into the circulation is not specific to the liver, and raised levels have been demonstrated in various situations where accumulation and/or degradation of type III collagen occur. Levels are high in children and adolescents and in pregnancy as a result of normal growth. Other reported causes of raised PIIINP include myelofibrosis, small cell carcinoma, breast carcinoma with bony metastases, ovarian carcinoma, bone fractures, burns and myocardial infarction. The physician must therefore always use clinical judgement to interpret the results appropriately [4]. The present study showed that TNF- α was significantly higher in psoriatic patients in comparison with control group in agreement with [17] and disagreement with [18]. Significant change in TNF- α was seen following low dose one month treatment with methotrexate in agreement with [19]. The significant rise of TNF- α in psoriatic patients is expected since the chronic inflammatory process involved the recruitment of macrophages by T-lymphocytes and these macrophages will secrete TNF- α in large amounts and it will cause keratinocyte proliferation. The absence of changes in the level of TNF- α following methotrexate treatment can be attributed to the mechanism of action of this drug which mainly resides on the inhibition of the enzyme tetrahydrofolate reductase and has no effect on TNF- α production by inflammatory cells

References

- [1] Basko-Plluka J.L., Petronic-Rosic V. (2012). Psoriasis: epidemiology, natural history, and differential diagnosis. *Psoriasis Targets Ther*; 2: 67–76
- [2] Danielsen, K., Olsen, A. O., Wilsgaard, T. and Furberg, A. S. (2013). Is the prevalence of psoriasis increasing? A 30-year follow-up of a population-based cohort. *Br J Dermatol.*; 168: 1303–1310.
- [3] Lowes, M., Bowcock, A. M. and Krueger, J. (2007). Pathogenesis and therapy of psoriasis. *Nature* 445, 866–873
- [4] Boffa, M. J., Smith, A. and Chalmers, R. J. G. (2009). Comment on: Liver fibrosis in patients with psoriasis and psoriatic arthritis on long-term, high cumulative dose methotrexate therapy. *Rheumatology*; 48:1464.
- [5] Brazzelli V, Grasso V, Fornara L, *et al.* (2010). Homocysteine, vitamin B12 and folic acid levels in

- psoriatic patients and correlation with disease severity. *Int J ImmunopatholPharmacol*; 23: 911-916.
- [6] Tobin A., Hughes R., Hand E. B. and Kirby B. (2011). Homocysteine status and cardiovascular risk factors in patients with psoriasis: A case-control study. *Clinical and Experimental Dermatology*; 36(1):19-23.
- [7] Ataseven A., Kesli R., Kurtipek G. S. and Ozturk P. (2014). Assessment of Lipocalin 2, Clusterin, Soluble Tumor Necrosis Factor Receptor-1, Interleukin-6, Homocysteine, and Uric Acid Levels in Patients with Psoriasis. *Disease Markers*; 2014: ID 541709, 7 pages.
- [8] Malerba M., Gisondi P., A. Radaeli, R. Sala, P. G. CalzavaraPinton, and G. Girolomoni.(2006). Plasma homocysteine and folate levels in patients with chronic plaque psoriasis. *British Journal of Dermatology*; 155(6): 1165–1169
- [9] McDonald I., Connolly M. and Tobin A. (2012).A Review of Psoriasis, aKnown Risk Factor for Cardiovascular Disease and Its Impact on Folate and Homocysteine Metabolism. *Journal of Nutrition and Metabolism* ;2012: ID 965385 4 pages.
- [10] Malekzad F., Qeisari M., Nasiri S., Rahmati-Roudsari M., Saedi M. and Sarlak M. (2009). Does Short-Term Low-Dose Methotrexate Treatment Affect Homocysteine Blood Level in Patients with Psoriasis? *Iranian Journal of Dermatology*; 12(1): 9-12.
- [11] Hornung N., Ellingsen T., Stengaard-Pedersen K., and Poulsen J. H. (2004).Folate, homocysteine, and cobalamin status in patients with rheumatoid arthritis treated with methotrexate, and the effect of low dose folic acid supplement. *The Journal of Rheumatology* ; 31 (12): 2374-2381.
- [12] Sommer D. M., S. Jenisch, M. Suchan, E. Christophers, and M. Weichenthal. (2006)., “Increased prevalence of the metabolic syndrome in patients with moderate to severe psoriasis, ” *Archives of Dermatological Research*; 298(7) :321–328.
- [13] Farrell G. C. andLarter C. Z. (2006)., “Nonalcoholic fatty liver disease: from steatosis to cirrhosis, ” *Hepatology*; 43 (2): S99–S112.
- [14] Maurice P. D. L., Maddox A. J., Green C. A., Tatnall F., Schofield J.K. and Stott D. J. (2005). Monitoring patients on methotrexate: hepatic fibrosis not seen in patients with normal serum assays of aminoterminal peptide of type III procollagen. *Br J Dermatol*; 152:450–457.
- [15] Lindsay K, Fraser AD, Layton A, Goodfield M, Gruss H, Gough A. (2009). Liver fibrosis in patients with psoriasis and psoriatic arthritis on long/term, high cumulative dose methotrexate therapy. *Rheumatology*; 48:569–72.
- [16] Chalmers R. J. G., Kirby B., Smith A., *et al.* (2005). Replacement of routine liver biopsy by procollagen III aminopeptide for monitoring patients with psoriasis receiving long-term methotrexate: a multicentre audit and health economic analysis. *British Journal of Dermatology*;152 (3):444–450.
- [17] Verghese B., Bhatnagar S., Tanwar R., Bhattacharjee J. (2011). Serum Cytokine Profile in Psoriasis-A Case–Control Study in a Tertiary Care Hospital from Northern India. *Ind J ClinBiochem*; 26(4):373–377.
- [18] Jacob SE, Nassiri M, Kerdel FA, Vincek V. (2003). Simultaneous measurement of multiple Th1 and Th2 serum cytokines in psoriasis and correlation with disease severity.*Mediat Inflamm.*;12(5):309–13.
- [19]Rodriguez-Jimenez N. A., Garcia-Gonzalez C. E., Lopez K. P.A., *et al.* (2014). Modifications in Lipid Levels Are Independent of Serum TNF- α in Rheumatoid Arthritis: Results of an Observational 24-Week Cohort Study Comparing Patients Receiving Etanercept Plus Methotrexate or Methotrexate as Monotherapy. *BioMed Research International* (2014): ID 510305, 10 pages.

Author Profile

Anwar H. Mathboob received the B.Sc Pharmacy from college Pharmacy/ Baghdad University.