Original Article

Effects of Hydroxychloroquine on markers of oxidative stress and antioxidant reserve in rheumatoid arthritis patients

Rawaa Jassim Ahmed[1], Zeina Abdulmunim Althanoon[2]*

[1]IBN-SINA Teaching Hospital, Nineveh Province, Mosul, Iraq. [2]Department of Pharmacology and Toxicology, College of Pharmacy, University of Mosul, Mosul, Iraq.

Correspondence: Zeina Abdulmunim Althanoon, Department of Pharmacology and Toxicology, College of Pharmacy, University of Mosul, Mosul, Iraq. dr.zeina@uomosul.edu.iq

ABSTRACT

Rheumatoid arthritis (RA) is a chronic condition which is a major contributor to disability in older adults. The cornerstone of rheumatoid arthritis care is now disease-modifying antirheumatic medication. The most widely used medication among these is hydroxychloroquine, which has been shown to have antioxidant properties and delay the course of the illness. Thus, the current study's objective is to compare the antioxidant and oxidant status of RA patients in Mosul City, Iraq, to that of healthy controls in order to assess the impact of hydroxychloroquine. For the study, a total of 80 people with rheumatoid arthritis and 80 people who appeared to be in good condition were enrolled. Patients with rheumatoid arthritis were further separated into groups I (those taking hydroxychloroquine) and II (those not taking hydroxychloroquine) (group II). All of the subjects had their lipid peroxidation index, malondialdehyde and total antioxidant activity, superoxide dismutase, and glutathione levels examined. Patients with arthritis treated with hydroxychloroquine had significantly higher levels of total antioxidant activity ($p = 0.048$) and lower levels of lipid peroxidation ($p = 0.04$) than patients who were not treated with hydroxychloroquine. Superoxide dismutase, an antioxidant enzyme, had a positive association with lipid peroxide levels, whereas MDA and total antioxidant activity had a significant negative correlation ($p=0.01$) in both patient groups. The results of this research recommend that hydroxychloroquine management for RA patients causes a reduction in oxidative stress which is a cardiovascular risk factor and increases some parameters of antioxidants (especially GSH-Px) in these patients.

Keywords: Rheumatoid arthritis, Hydroxychloroquine, Malondialdehyde, Glutathione

Introduction

The cause of the chronic systemic autoimmune disease rheumatoid arthritis (RA) is uncertain. The care of RA patients in the Middle East might be optimized, according to information first from the area [1]. In addition to publications from EULAR and the American College of Rheumatology (ACR), Iraqi rheumatologists also refer to unofficially disclosed governmental recommendations for the management of RA that have the Ministry of Health in Iraq's endorsement [2]. An all-encompassing word for joint inflammation is arthritis. Osteoarthritis (OA) and RA are two separate types of arthritis, though [3].

RA and OA both have an impact on the joints, but they are completely different manifestations of the same general condition [4]. A chronic inflammatory (nonsuppurative inflammation) illness involving many collateral joints primarily characterizes RA's clinical presentation. A vascular injury that is not limited to the joints may also accompany it and result in epidermal lumps, pericarditis, and myocarditis [5].

The osteoarthritic of the joint, that subsequently migrate to articular cartilage, bone tissue, joint ligaments, and tendons, is the primary pathogenic hallmark of RA. then an extensive inflammation of connective tissues is seen, such as serosa, heart, lung, and eyes [6]. Joint deformities, such as synovial inflammation, exudation, cell proliferation, granuloma development, cartilage, and bone tissue loss, and ultimately joint inflammation and malfunction, may be present immediately when the illness affects both cartilage and bone [7, 8].

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Ahmed RJ, Althanoon ZA. Effects of Hydroxychloroquine on markers of oxidative stress and antioxidant reserve in rheumatoid arthritis patients. J Adv Pharm Educ Res. 2022;12(3):65-71. https://doi.org/10.51847/7wv6zqfhb5

Joint cartilage loss is linked to aberrant cytokine production, and the absence of balance that exists among the protective and damaging cytokines is regarded as the pathophysiology of RA. Additionally, immune-inflammatory cells and inflammatory chemokines work together to accelerate the pathogenic course of RA [9].

The present research proves that besides inflammation, oxidative stress products likewise have a crucial role in the progression of pathogenesis and pathological of RA [10]. Oxidative stress is The lack of balance between the production of free radical/reactive oxygen species (ROS) and antioxidant defenses, preferring prooxidants. Too many free radicals produced by oxidative stress will lead to the oxidation of several substances within the body [11, 12]. The level of malondialdehyde (MDA) rises in RA patients' bodies due to excessive reactive oxygen species, and the superoxide dismutase (SOD) system is disrupted, which weakens the antioxidant of the body defenses as well as exacerbates bone loss [13]. Additionally, oxidative stress and the energy metabolism of synovial tissue in RA individuals are tightly related [14]. Studies investigating the link between oxidative stress, SOD antioxidation, and monitoring in RA patients might consequently disclose the pathogenic process of RA and lead to the discovery of novel anti-rheumatoid arthritis medications [15-19]. Therefore, this study involves hydroxychloroquine management of RA against oxidative stress [20, 21].

Currently, numerous trials using treatments for oxidative stress have been made [22, 23]. It is impossible to [provide practicing Doctors as well as individuals with RA with literature and treatment options for the pathogenic mechanism of oxidative stress due to the diversity of these clinical trials interventions and results, as well as the inconsistent quality and degrees of the evidence, supplied [20-23].

Materials and Methods

Ninety (Male/Female; aged 30 to 65), made up the studied groups. These patients were chosen from the IBN-SINA Teaching Hospital's rheumatology clinic and other hospitals in Mosul. The individuals were all diagnosed with RA based on the most recent ACR guidelines. The sickness lasted an average of 4.7 years. As controls, people of the same sex and age were chosen. The aim of this research was enumerated to all participants, and consent was attained in writing. Patients taking NSAIDs for pain management were not allowed to participate in the trial, nor were those taking corticosteroids or experiencing an acute deterioration of their illness. The project received the project's approval from the institutional ethics committee.

The Study group of RA patients was grouped as follows:

Group I – consisted of 40 RA patients treated with Hydroxychloroquine.

Group II - consisted of 40 RA patients not treated with Hydroxychloroquine / DMARDs.

blood was collected from participants, Plasma MDA was measured [24] and total antioxidant activity (TAA) [25, 26]. Superoxide Dismutase (SOD) [27, 28] and whole blood glutathione (GSH) [29]. Data were statistically analyzed by Unpaired t-test and Pearson's Correlation and expressed in terms of p value [30, 31].

A commercial kit was used to estimate the serum concentration of MDA for the total level of MDA (USCN Life Science Inc., Houston, USA). On a plate reader STAT FAX 2100, the results were read spectrophotometrically at 450 nm (Awareness Technology, Palm City, Florida, USA). The amount of MDA that was detected was measured in nanograms per milliliter (ng/mL). The Windows version 12.6.1.0 of the MedCalc software was used to conduct statistical analysis. To check whether the data had a normal distribution, the Kolmogorov-Smirnov or Shapiro-Wilk tests were utilized. When comparing variables consisting of a normal distribution, the t-test for independent samples was utilized in showing the data as the mean standard deviation. The Mann-Whitney U-test was used to analyze the median and interquartile range for variables that did not have a normal distribution. For the statistical analysis of more than three groups, ANOVA and the Kruskal-Wallis test were utilized.

Results and Discussion

Table 1 Shows the results of the comparison of mean oxidative stress parameters (Malondialdehyde (MDA), total antioxidant status (TAOS)) of RA patients between group I, group II and group III. There are highly significant differences in MDA parameters among group I, group II and group III (control) (p= 0.001).

Table 1. Comparison in oxidative stress parameters among the three groups

Studied parameters	Group I	Group II	Control	P-value*
MDA (ng\ml)	**105.7 ± 57.5^{B}**	**237.8 ± 108.9^{A}**	**22.1 ± 18.31^{C}**	**0.001**
TAOS (U/ml)	**1.257 ± 1.20^{A}**	**1.150 ± 1.01^{A}**	**1.133 ± 0.872^{A}**	**0.882**

* One-way ANOVA-test with Tukey's Pair wise comparisons was applied. Means that do not share a letter are significantly different.Data expressed as mean±SD

MDA (p = 0.0001) was elevated in RA individuals in comparison to controls. Whole blood GSH (p =0.005) & total antioxidant activity levels (p = 0.0001) were reduced in RA individuals in comparison to controls. Superoxide dismutase activity (p=0.005) was remarkably increased in RA individuals in comparison to controls.

Figure 1 Show highly significant differences in MDA among the three groups (group I, group II, group III), (p= 0.001).

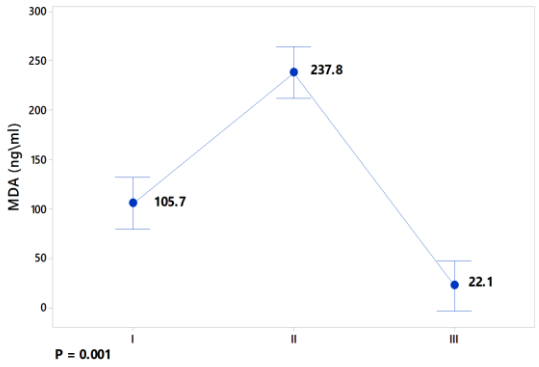

Figure 1. The difference in MDA among the three groups.

Table 2 Shows the results of the comparison mean serum lipid profile (cholesterol, TG, HDL, LDL, VLDL, Atherogenic index (AI)) of RA patients between group I, and group II and group III. There are highly significant differences in TG among group I, group II and group III (control) (p= 0.027).

Table 2. Comparison in lipid profile parameters among the three groups

Lipid profile	Group I	Group II	Control	P-value*
Cholesterol (mg\dl)	195.70±38.24^{A}	184.47±30.89^{A}	175.07±36.02^{A}	0.081
TG (mg\dl)	148.3±69.8^{A}	119.37±40.8^{AB}	114.53±37.71^{B}	0.027
HDL (mg\dl)	48.87±8.26^{A}	49.13±7.18^{A}	52.93±6.95^{A}	0.068
LDL (mg\dl)	114.39±38.88^{A}	110.0±35.83^{A}	99.23±40.63^{A}	0.296
VLDL (mg\dl)	29.65±13.96^{A}	25.32±12.04^{A}	22.90±7.43^{A}	0.075
Atherogenic Index	4.08±1.423^{A}	3.86±1.098^{A}	3.40±1.16^{A}	0.098

* One-way ANOVA-test with Tukey's Pair wise comparisons was applied. Means that do not share a letter are significantly different.

Figure 2 Show highly significant differences in TG among the three groups (group I, group II, group III), (p= 0.027).

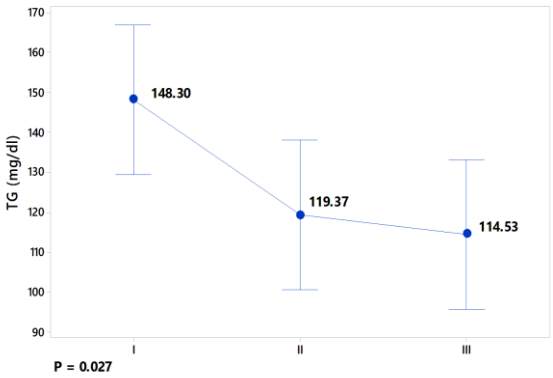

Figure 2. The difference in TG among the three groups.

Table 3 Show the results of Correlation matrix between different parameters (CRP, IL-1β, IL-6, TNF-α, MDA, TAOS, cholesterol, TG, HDL, LDL, VLDL, Atherogenic index) in group I. There are statistically significant positive correlation (r= 0.458)(p-value= 0.011) between IL-6β and TNF-α, statistically significant positive correlation (r=0.456) (p-value= 0.011) between MDA and TNF-α, statistically significant positive correlation (r=0.390)(p-value=0.033) between IL-1β and cholesterol, statistically significant positive correlation (r=0.474) (p-value=0.008) between IL-1β and TG, statistically significant positive correlation (r= 0.485) (p-value= 0.006) between IL-1β and VLDL and statistically significant negative correlation (r = -0.385) (p-value= 0.036) between TAOC and TG in the group I.

Figure 3 Show statistically significant negative correlation (r = -0.385) (p-value= 0.036) between TAOC and TG in the group I.

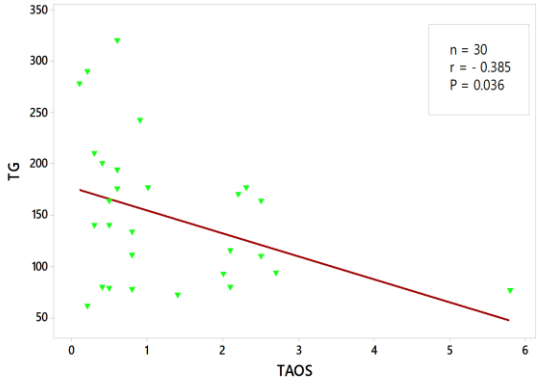

Figure 3. Correlation between TAOC and TG in the group I.

The joints are affected by the chronic inflammatory illness known as rheumatoid arthritis (RA). A gradual symmetrical inflammation of the afflicted joints, which leads to cartilage loss, bone erosion, and incapacity, are its defining features [32]. Early on, only a few joints are afflicted; however, as the disease progresses, many more joints are damaged, and extra-articular symptoms are frequently reported [33]. Although there is no known cause of RA, both genetic and environmental variables have been demonstrated to have a role in its onset [34].

The present study was performed to evaluate the effect of hydroxychloroquine treatment on oxidants and antioxidant status {Malondialdehyde (MDA), total antioxidant status (TAOS)} and lipid profile {cholesterol, TG, HDL, LDL, VLDL, Atherogenic index (AI)}.

The current study involved sixty patients whose ages between (20-60) years of both sexes, known to have RA and referred by rheumatologists enrolled in this study.

The patients were separated into 2 groups: the first group (group I) included, thirty patients using conventional treatment (methotrexate, (MTX) and prednisolone) plus hydroxychloroquine (HCQ), Plaquenil tablet manufactured by

Sanofi Aventis U.S., Bridgewater, UK, at dose 400 mg once daily.

The second group (group II) included thirty patients on conventional treatment (methotrexate, (MTX) and prednisolone) without using hydroxychloroquine. Both treatment groups had a duration of therapies of at least 6 months. The efficacy of both therapies on the clinical course of the disease was assessed by using Disease Activity Score-28 (DAS-28). As a control group III, 30 healthy, non-smokers, age and sex-matched to the patient, were considered).

Table 3. Correlation matrix between different parameters in Group I, [n = 30]

Items	Correl coef*	ESR	CRP	IL-1	IL-6	TNF-α	MDA	T-AOS	Chol	TG	HDL	LDL	VLDL
CRP	r	- 0.396	---	---	---	---	---	---	---	---	---	---	---
	P	0.031	---	---	---	---	---	---	---	---	---	---	---
IL-1	r	0.030	- 0.060	---	---	---	---	---	---	---	---	---	---
	P	0.877	0.752	---	---	---	---	---	---	---	---	---	---
IL-6	r	- 0.125	0.154	0.024	---	---	---	---	---	---	---	---	---
	P	0.512	0.417	0.898	---	---	---	---	---	---	---	---	---
TNF-α	r	- 0.033	0.255	0.178	0.458	---	---	---	---	---	---	---	---
	P	0.864	0.173	0.348	0.011	---	---	---	---	---	---	---	---
MDA	r	- 0.009	0.167	0.229	0.229	0.496	---	---	---	---	---	---	---
	P	0.963	0.378	0.223	0.223	0.005	---	---	---	---	---	---	---
T-AOS	r	- 0.172	0.158	- 0.153	0.055	- 0.093	0.282	---	---	---	---	---	---
	P	0.364	0.404	0.418	0.772	0.626	0.131	---	---	---	---	---	---
Chol	r	0.109	- 0.139	0.390	0.099	0.103	0.244	-0.171	---	---	---	---	---
	P	0.565	0.464	0.033	0.601	0.588	0.194	0.365	---	---	---	---	---
TG	r	0.074	- 0.060	0.474	0.049	0.099	-0.021	- 0.385	0.432	---	---	---	---
	P	0.698	0.755	0.008	0.798	0.605	0.912	0.036	0.017	---	---	---	---
HDL	r	- 0.166	0.269	- 0.149	0.159	0.135	0.079	0.284	- 0.694	- 0.599	---	---	---
	P	0.380	0.151	0.433	0.400	0.478	0.677	0.128	0.001	0.001	---	---	---
LDL	r	0.151	- 0.136	0.302	0.092	0.005	0.261	- 0.044	0.896	0.203	- 0.618	---	---
	P	0.426	0.475	0.104	0.629	0.979	0.163	0.819	0.001	0.281	0.001	---	---
VLDL	r	0.074	- 0.060	0.485	0.049	0.099	- 0.021	- 0.345	0.432	1.000	-0.599	0.203	---
	P	0.698	0.755	0.006	0.798	0.605	0.912	0.033	0.017	*	0.001	0.281	---
AI	r	0.020	- 0.168	0.361	- 0.075	0.043	0.185	- 0.222	0.850	0.421	- 0.847	0.786	0.421
	P	0.916	0.374	0.040	0.695	0.820	0.329	0.238	0.001	0.021	0.001	0.001	0.021

* Pearson correlation method (r) was used.

The current results show that the results of comparison of mean oxidative stress parameters (Malondialdehyde (MDA) {(105.7 ± 57.5), (237.8 ± 108.9),(22.1 ± 18.31)}, total antioxidant status (TAOS) {(1.257 ± 1.20), (1.150 ± 1.01), (1.133 ± 0.872)}), are highly significant differences in MDA parameter among group I, group II and group III (control) respectively (p= 0.001) with non -significant differences in TAOS among group I, group II and group III (p= 0.882). Also, this study shows significant differences in MDA levels between RA patients in group I and group II. Salem & Zahran, 2021 agreed with the current study about the MDA parameter and showed highly significant differences in MDA levels between RA patients with or without taking HCQ while disagreeing about TAOS, which also showed highly significant differences in TAOS between groups. Le Zhang *et al.*, 2020 disagreed with the current study and showed non-significant differences in MDA levels in RA patients with or without taking HCQ. While Nuttall *et al.*, 2003: Spadaro *et al.*, 2006: Firuzi *et al.*, 2006 agreed with the current study, and showed non-significant differences in TAOS levels in RA patients with or without taking HCQ.

The current study shows that the results of the comparison mean serum lipid profile of RA patients between group I, group II and group III respectively, there are highly significant differences in TG among group I, group II and group III (p= 0.027) and highly significant differences in TG between group I and group II.

Salem and Zahran, 2021 showed highly significant differences in lipid profile(total cholesterol, TG, HDL, LDL, VLDL, AI) in RA patients with or without taking HCQ. This study agreed with the current study about TG while disagreeing with the current study regarding other parameters of lipid profile [35]. Nuttall *et al.*, 2003 showed non-significant differences in lipid profile (total cholesterol, TG, HDL, LDL, VLDL, AI) in RA patients with or without taking HCQ. This study did not agree with the current study about TG while agreed with the current study regarding other parameters of lipid profile [36].

The results of the Correlation matrix between different parameters (CRP, IL-1β, IL-6, TNF-α, MDA, TAOS, cholesterol, TG, HDL, LDL, VLDL, Atherogenic index) in group I. There are statistically significant positive correlation (r= 0.458) (p-value= 0.011) between IL-6 and TNF-α. Jang *et al.*, 2006 agreed with the current study and showed a significant positive correlation between IL-6 and TNF-α [37]. Samimi *et al.*, 2020 disagreed with the current study and showed no correlation between IL-6 and TNF-α [38]. The current study shows statistically significant positive correlation (r= 0.456) (p-value= 0.011) between MDA and TNF-α. Abdollahzad *et al.*, 2015 and Salem *et al.*, 2021 agreed with the current study and showed a significant positive correlation between MDA and TNF-α [39, 40], while Gasmi *et al.*, 2021 disagreed with the current study and showed no correlation between MDA and TNF-α [41].

The current study showed a positive correlation between IL-1β and cholesterol, TG, VLD versus IL-1β, and a negative correlation between TAOS and TG in group I. The effects of HCQ on MHC Class II expression and antigen demonstration, activities of pro-inflammatory cytokines [such as interleukin-1 (IL-1) tumor necrosis factor-(TNF)], control of toll-like receptor-9 stimulation, and leucocyte generation of reactive oxygen species (ROS), i.e. antioxidant capacity (TAOS) [42] were demonstrated by Rainsford *et al.* in 2015.

Ben-Zvi *et al.*, 2012 [43] showed, In addition, it was demonstrated that hydroxychloroquine (HCQ) medication reduced triglyceride levels in RA patients regardless of concurrent steroid use, while Tam *et al.*, 2000 shows HCQ had no significant effect on the serum lipid profile [44]. Finally, the current study shows that HCQ was a highly significant improvement in oxidative stress parameter (MDA), and TG concentrations in the serum patients with RA compared with RA patients on conventional therapy and control and was a highly significant improvement in MDA and TG levels in comparison with conventional therapy.

It is worthy to mention that hydroxychloroquine is used in COVID-19 with an obscure mechanism of action [45-49], either alone or with zinc as a cofactor for potential therapy [50]. While the proposed mechanism also involved reduced oxidative and pro-inflammatory damage, nonetheless, these data need further confirmation at a molecular level to confirm the therapeutic activities.

Conclusion

The addition of hydroxychloroquine to the regimen of patients with rheumatoid arthritis improved the outcome via reducing metabolic derangement and restoration of redox quasi-equilibrium.

Acknowledgments: We thank the regional research ethics committees at the University of Mosul, College of Pharmacy, and Health Administration Research Technical Support for their Ethics approval of the study. We are also very grateful and express our deep thanks to Dr.Gaidaa A.Ahmed(Consultant Rheumatologist) and to all of the medical staff of the in-patient and out-patient clinic in IBN-SINA Teaching Hospital in Mosul city for their valuable help and support in carrying out the requirement of the research study.

Conflict of interest: No potential conflicts exist. We had full access to all the information in the study and take full responsibility for the integrity of the information and the accuracy of the data analysis.

Financial support: The research was fully self-funded.

Ethics statement: The study was approved by the Research Ethical Committee and Scientific Committee in the College of Medicine/University of Mosul, Study approval letter CoM/UoM/4109 on 29.06.2021.

References

1. Radu AF, Bungau SG. Management of rheumatoid arthritis: an overview. Cells. 2021;10(11):2857.
2. Al-Ezzi MY, Pathak N, Tappuni AR, Khan KS. Primary Sjögren's syndrome impact on smell, taste, sexuality and quality of life in female patients: a systematic review and meta-analysis. Mod Rheumatol. 2017;27(4):623-9.
3. Schmidt T, Berthold E, Arve-Butler S, Gullstrand B, Mossberg A, Kahn F, et al. Children with oligoarticular juvenile idiopathic arthritis have skewed synovial monocyte polarization pattern with functional impairment—a distinct inflammatory pattern for oligoarticular juvenile arthritis. Arthritis Res Ther. 2020;22(1):1-3.
4. Alkhatib MH, Alyamani SA, Abdu F. Incorporation of methotrexate into coconut oil nanoemulsion potentiates its antiproliferation activity and attenuates its oxidative stress. Drug Deliv. 2020;27(1):422-30.
5. Araújo CL, Salles BC, Duarte SM, Rodrigues MR, Paula FB. Passion fruit (Passiflora edulis) leaf extract modulates the oxidative metabolism of rat peritoneal neutrophils in a model of inflammation. Braz J Pharm Sci. 2020;56.
6. Lodhi AH, Furwa K, Madni A. Role of oxidative stress and reduced endogenous hydrogen sulfide in diabetic nephropathy. Drug Des Devel Ther. 2021;15:1031.
7. Althanoon AZ. Evaluation of the therapeutic effects of combined hydroxychloroquine and azithromycine in patients with Covid-19. Mmsl. 2022;91(3).
8. Mawahib C, Nabila Z, Nabila S, Chawki B, Salah A. LC-MS analysis, antioxidant and alpha-glucosidase inhibitory activities of Centaurea papposa extracts. Bangladesh J Pharmacol. 2019;14(4):159-65.
9. Younus M, Hasan MM, Ahmad K, Sharif A, Asif HM, Aslam MR, et al. α-Glucosidase Inhibitory, Anti-Oxidant, and Anti-Hyperglycemic Effects of Euphorbia nivulia–Ham. in STZ-Induced Diabetic Rats. Dose-Response. 2020;18(3):1559325820939429.

10. Althanoon Z, Faisal IM, Ahmad AA, Merkhan MM. Pharmacological aspects of statins are relevant to their structural and physicochemical properties. Syst Rev Pharm. 2020;11(7):167-71.
11. Al-Talib WH, Althanoon ZA. Effects Of Deferasirox Therapy On High Sensitivity C-Reactive Protein and Oxidative Stress Markers In Iron Overloaded, Beta-Thalassemic Patients. Syst Rev Pharm. 2020;11(12):1060-7.
12. Alkazaz AA, Faisal I, Merkhan M, Al-mukhtar H, Khalaf M, Zainal A, et al. Efficacy of drugs for classical trigeminal neuralgia; statistical study comparative to gold-standard carbamazepine. J Gar Univ. 2019;6(SCAPAS Conferance):194-200.
13. Merkhan MM, Abdullah E, Althanoon Z. Effect of Esomeprazole on serum creatinine and urea in patients with Peptic Ulcer. Res J Pharm Technol. 2022;15(1):160-4.
14. Al-Thanoon ZA, Mahmood IH. Effects of losartan vs. enalapril on the markers of metabolic syndrome. Oman Med J. 2012;27(1):27.
15. Althanoon ZA, Alkazzaz NA. Comparison of The Effects Of Deferasirox And Deferoxamine On Uric Acid And Renal Function In Patients with Beta Thalassemia. Syst Rev Pharm. 2020;11(11):214-22.
16. Althanoon ZA, Altalib WH, Khalaf MM. The Effects Of Vitamin E Supplementation In Patients With Beta Thalassemia On Iron Chelation TherapyNat Volatiles Essent Oils J. 2021:11516-28.
17. Althanoon ZA. Metabolic and Hormonal Effects Of Rosiglitazone In Overweight Women With PCOS. Pakistan J Med Health Sci. 2021;15(3):996-1000.
18. Althanoon ZA. Metabolic Adverse Effects of Antipsychotic Drugs in Patients with Schizophrenia. Clin Schizophr Relat Psychoses. 2021;15(6):1-7.
19. Althanoon ZA, Mahmood IH. Effect of lisinopril therapy on serum leptin, oxidative stress and C-reactive protein in hypertensive patients. Pharmacia. 2021;68:705.
20. Al-Khyate M, Althanoon ZA, Alkazaz NA. Adherence and Satisfaction of Deferasiroxversus Deferoxamine in Transfusion-Dependent Beta-Thalassemia. Indian J Forensic Med Toxicol. 2020;14(4).
21. Merkhan MM, Althanoon ZA, Mohammed AA, Faisal IM. Assessment of Pesticide Biohazards in Neurodegenerative Diseases; Data Analysis Statistical Study. Indian J Forensic Med Toxicol. 2021;15(4):1783.
22. Badr SA, Althanoon ZA. A review of the effect of Coenzyme Q10 in patients with polycystic ovary syndrome. Iraqi J Pharm. 2021;18(1):108-17.
23. Duan D, Wang Y, Pan D, Jin X, Yan Y, Song P, et al. Rheumatoid arthritis drug sinomenine induces apoptosis of cervical tumor cells by targeting thioredoxin reductase in vitro and in vivo. Bioorg Chem. 2022;122:105711.
24. Phull AR, Nasir B, ul Haq I, Kim SJ. Oxidative stress, consequences and ROS mediated cellular signaling in rheumatoid arthritis. Chem Biol Interact. 2018;281:121-36.
25. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 2001;54(5):356-61.
26. Niero G, Costa A, Franzoi M, Visentin G, Cassandro M, De Marchi M, et al. Genetic and non-genetic variation of milk total antioxidant activity predicted from mid-infrared spectra in holstein cows. Animals. 2020;10(12):2372.
27. Beauchamp CO, Fridovich I. Isozymes of superoxide dismutase from wheat germ. Biochim Biophys Acta (BBA)-Protein Structure. 1973;317(1):50-64.
28. Flohe L. [10] Superoxide dismutase assays. InMethods in enzymology 1984 Jan 1 (Vol. 105, pp. 93-104). Academic press.
29. Beutler E, Kelly BM. The effect of sodium nitrite on red cell GSH. Experientia. 1963;19(2):96-7.
30. Lynch CP, Cha ED, Mohan S, Geoghegan CE, Jadczak CN, Singh K. Two-year validation and minimal clinically important difference of the Veterans RAND 12 Item Health Survey Physical Component Score in patients undergoing minimally invasive transforaminal lumbar interbody fusion. J Neurosurg Spine. 2021;36(5):731-40.
31. Bland JM, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;327(8476):307-10.
32. Hayer S, Bauer G, Willburger M, Sinn K, Alasti F, Plasenzotti R, et al. Cartilage damage and bone erosion are more prominent determinants of functional impairment in longstanding experimental arthritis than synovial inflammation. Dis Model Mech. 2016;9(11):1329-38.
33. Littlejohn EA, Monrad SU. Early diagnosis and treatment of rheumatoid arthritis. Prim Care Clin Off Pract. 2018;45(2):237-55.
34. Deane KD, Holers VM. The natural history of rheumatoid arthritis. Clin Ther. 2019;41(7):1256-69.
35. Salem HR, Zahran ES. Vascular cell adhesion molecule-1 in rheumatoid arthritis patients. Relation to disease activity, oxidative stress, and systemic inflammation. 2021;42:620-8.
36. Nuttall SL, Heaton S, Piper MK, Martin U, Gordon C. Cardiovascular risk in systemic lupus erythematosus—evidence of increased oxidative stress and dyslipidaemia. Rheumatology. 2003;42(6):758-62.
37. Jang CH, Choi JH, Byun MS, Jue DM. Chloroquine inhibits production of TNF-α, IL-1β and IL-6 from lipopolysaccharide-stimulated human monocytes/macrophages by different modes. Rheumatology. 2006;45(6):703-10.
38. Samimi Z, Kardideh B, Chalabi M, Zafari P, Taghadosi M. The plasma Tumor Necrosis Factor-α (TNF-α) does not have any correlation with disease activity in rheumatoid arthritis patients treated with disease modifying anti-rheumatic drugs (DMARDs). Braz J Pharm Sci. 2020;56.
39. Abdollahzad H, Aghdashi MA, Jafarabadi MA, Alipour B. Effects of coenzyme Q10 supplementation on inflammatory cytokines (TNF-α, IL-6) and oxidative stress in rheumatoid

arthritis patients: a randomized controlled trial. Arch Med Res. 2015;46(7):527-33.

40. Salem AZ, Medhat D, Fathy SA, Mohamed MR, El-Khayat Z, El-Daly SM. Indole glucosinolates exhibit anti-inflammatory effects on Ehrlich ascites carcinoma cells through modulation of inflammatory markers and miRNAs. Mol Biol Rep. 2021;48(10):6845-55.
41. Gasmi A, Peana M, Noor S, Lysiuk R, Menzel A, Gasmi Benahmed A, et al. Chloroquine and hydroxychloroquine in the treatment of COVID-19: the never-ending story. Appl Microbiol Biotechnol. 2021;105(4):1333-43.
42. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. Inflammopharmacology. 2015;23(5):231-69.
43. Ben-Zvi I, Kivity S, Langevitz P, Shoenfeld Y. Hydroxychloroquine: from malaria to autoimmunity. Clin Rev Allergy Immunol. 2012;42(2):145-53.
44. Tam LS, Gladman DD, Hallett DC, Rahman P, Urowitz MB. Effect of antimalarial agents on the fasting lipid profile in systemic lupus erythematosus. J Rheumatol. 2000;27(9):2142-5.
45. Darweesh O, Abdulrazzaq GM, Al-Zidan RN, Bebane P, Merkhan M, Aldabbagh R, et al. Evaluation of the Pharmacologic Treatment of COVID-19 Pandemic in Iraq. Curr Pharmacol Rep. 2021;7(4):171-8.
46. Merkhan MM, Abdulrazzaq GM, Al-Taii HA. Coronavirus (COVID-19): preventive measures and potential interventions. Eur J Mol Clin Med. 2021;7(10):2020.
47. Gorial FI, Abdulrahman Younis A, Alkazzaz A, Arif Maroof AM, Qaradaghi TA, Mahmood CH, et al. COVID-19 among a sample of iraqi patients with rheumatic diseases: a multicenter study. MMSL. 2021;91(2):89-97.
48. Alsaaty MH, Alyouzbaki AZ, Younis WT. The impact of smoking on severity and outcome in patients with COVID-19 infection in Mosul city. Authorea Prepr. 2021;91(2):98-104.
49. Zainal AA, Merkhan MM. Impact of antidiabetic drugs on risk and outcome of covid-19 infection: a review. MMSL. 2022;91(2):140-60.
50. Althanoon ZA, Merkhan MM. Effects of zinc supplementation on metabolic status in patients with metabolic syndrome. Acta Pol Pharm. 2021;78(4):521-6.