# STATUS OF LIPID PEROXIDATION BY PRODUCTS; MALONDIALDEHYDE AND URIC ACID IN DIABETES MELLITUS (TYPES 2 )AND RHEUMATOID ARTHRITIS

## ( A COMPARATIVE STUDY)

**Hiba abdul-Atheem* , Ahmed A.Naser Al-Amiry**,Moayed Naji Majeed*****

## ABSTRACT:

The aim of the current study was to compare antioxidant defenses and oxidative stress markers in patients with Diabetes Mellitus (DM) and in those with Rheumatoid Arthritis (RA). Across-sectional study was conducted with 74 participants in two distinct groups: Diabetes Mellitus types 2, Rheumatoid Arthritis and healthy blood donors. Malondialdehyde and uric acid were compared between these groups. Uric acid was measured as antioxidant defenses tested in blood samples from (RA) and (DM), rather than the degree of lipid peroxidation was measured in term of malondialdehyde (MDA). MDA level was 3.29 ± 0.59 µmol/L in control group which was significantly lower than in diabetic and Rheumatoid arthritis patients 6.32 ± 0.84 µmol/L , 6.75 ± 0.77 µmol/L respectively with ($p < 0.001$). Moreover; concentrations of UA in DM was 3.65 ± 0.11 mg/dl significantly lower than in control 4.56 ± 0.29 mg/dl with ($p < 0.001$),conversely, we found that no significant change in the level of UA in RA 4.23 ± 0.66 mg/dl, compared with health subjects 4.56 ± 0.28 mg/dl. These results suggest that the feasibility to find out a new evidences for a possible relationship between these pathologies can be found.

## INTRODUCTION:

In recent years, a great number of studies have investigated the possible role of Reactive Oxygen Species (ROS) in the etiology and pathogenesis of several diseases[1]. The effects of lipid peroxidation in biological systems have been described in the development of Diabetes Mellitus (DM) [1-5], and in Rheumatoid Arthritis (RA)[6-9]. Type 2 diabetes mellitus (DM) is a major global health problem that affects over 200 million individuals worldwide[10].The increased oxidative stress in DM contributes to the development of diabetic complications. Oxygen derived free radicals and reactive oxygen species interact with the lipid bilayer of the cell membrane resulting in lipid peroxidation. Malondialdehyde (MDA) is a stable end product of lipid peroxidation. Elevated MDA levels alter the structural integrity of the cell membranes[11].Moreover, the overproduction of ROS due to persistent hyperglycemia produces oxidative protein damage, which would be related to the pathogenesis of diabetic' complications[12]. Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, joint tenderness, and

* College of medicine ,dep.of Biochemistry,University of Thi-Qar.
** College of medicine ,dep.of physiology,University of Thi-Qar.
***College of medicine ,dep.of pediatrics,University of Thi-Qar.

destruction of synovial joints, leading to severe disability and premature mortality[13-17]. Formation of reactive oxygen species (ROS) and lipid peroxides as a result of disease activity may play a role in perpetuate in local inflammatory reactions in the joint in RA[18]. Synovial fluid from rheumatoid patients contains products of lipid peroxidation that correlate with considerably increase the probability of disease severity as was measured clinically and in experimental systems[19.] Furthermore, there are many evidences that chronic systemic inflammation predispose to development of DM. The aim of present study to compare antioxidant defenses and oxidative stress markers in patients with Diabetes Mellitus (DM) and in those with Rheumatoid Arthritis (RA) in order to find out if new evidences for a possible relationship between these pathologies can be found.

# MATERIALS & METHODS:

This study was conducted at AL-Hussein Teaching Hospital in Thi-qar governorate.

**Healthy Subjects:** A total number of 30 subjects were taken as a control group , their age was ranging from 20 to 50 years (mean+/-SD:36.4+/-4.6) all were normal glycemic (FPG, mean+/-SD=93.3+/-5.33 mg/dl) , ESR , RF and C-reactive protein were normal during laboratory investigation.

**Diabetic patient:** Twenty two patients with type 2 DM(NIDDM) were enrolled in this study. their age was from 33 to 60 years (mean+/-SD=45.3+/-6.3) with their mean value of fasting plasma glucose (FPG, mean+/-SD:157.36+/-mg/dl) and the duration of the disease is from 2 to 10 years. Medical history was taken regarding age, gender, duration of illness, type of treatment, history of other illness and smoking.

**Rheumatoid Arthritis**: Twenty two patients (8 males and14 female patients) with age mean range of 58 ± 4 years. All the patients satisfied the 1987 American College of Rheumatology criteria for the diagnosis of RA and had rheumatoid factor positive. Those patients who had been receiving corticosteroid agents. However, patients who had been receiving ordinary dosages of none steroidal anti-inflammatory drugs (NSAID) were not excluded.

**Blood Sampling:** Blood samples with and without EDTA as anticoagulant were with-drawn from both the patients and the controls. sera were immediately separated by centrifugation at 3000 rpm for 5 min.

**Lipid peroxidation:** For quantitative evaluation of lipid peroxides, there transformation into coloured compound under the effect of thiobarbituric acid (TBA) was used. MDA, the final product of fatty acids peroxidation, react with TBA with the formation of a coloured, which was determined spectrophotometrically (*APEL* spectrophotometer) at a wave-length 530nm according to Satoh[20]**.**

**Uric acid (UA)** :Using a commercially available kit BioLabo ,France.

**C-reactive protein** (**CRP)** was determined by immuneoturbidimetric technique (Schiapparell Biosy stems, the Netherlands). The level of rheumatoid factor (RF) was studied measured by nephelometric method (BNII, Dade Behring, Germany).

# RESULTS & DISCUSSION:

A total of 74 subjects were enrolled in this study, including 30 healthy controls and 44 patients (was dividing into 2 groups, The first group of twenty two patient with type 2 DM(NIDDM) and the second group of 22 patients the diagnosis of rheumatoid Arthritis). It is known that during health, ROS production is low and lipid peroxidation is inhibited by the combined activities of the antioxidant systems present in the plasma. The loss of the normal oxidant–antioxidant equilibrium may either be due to a decreased antioxidant or to an increased generation of oxidants[21].

**Lipid peroxidation by product; Malondialdehyde in Rheumatoid Arthritis and Diabetic patients:**

In the comparison, the level of MDA was (3.53+/-0.80)Mmol/L in control group which was significantly lower than diabetic and Rheumatoid arthritis (6.30+/-1.01 Mmol/L),(7.20+/-0.60 Mmol/L) respectivly as show in fig(1 ). *In DM* (*type 2*) *patients :*this increment in lipid peroxidation may be attributed to many factors, we think that hyperglycemic induces alteration in intracellular mechanism which yield higher amount of free radicals leading to imbalance between the free radicals and antioxidant equilibrium and this, in turn can caused augmentation in lipid peroxidation detected by increment in level of MDA. Our observation is in agreement with several study[22,23,24] . *In RA* :a chronic immune-inflammatory multisystem disease, the polymorphonuclear leukocytes are activated and ROS are generated in excessive amounts[38].This enhanced oxidation plays a significant role in tissue damage and chronic inflammation process[25].In both pathologies, these increased ROS concentrations cause lipid peroxidation, leading to toxic damage of tissues. Our results showed that although patients with DM has increased the level of MDA , in RA this increase was higher. These results indicated that the overproduction of ROS in RA was more important than that in DM .

**Uric Acid level in Rheumatoid Arthritis and Diabetic patients:**

This study showed that the level of UA was 4.56±0.29 mg/l in control group, which is significantly lower in diabetic patient (3.65±0.11mg/l) with ($p<0.05$) (fig. 2), conversely, we found that no significant decrease was found in the level of UA between controls and RA patient, (4.23±0.66mg/l), (4.8+/-0.3mg/l) are shown in **table 1.**

*In DM*(*type 2*) *patients*: We think that hyperglycemia can induce this reduction in the uric acid level due to its osmotic diuresies mechanism. Same observation were reported by many study,[26,27,28].In addition, Sinclair et al (ref.29) had regard the principle mechanism for reducing UA in diabetic is due to hyperglycemia which cause a reduction of the antioxidants system and increase in oxidative stress. *In RA:* Uric acid has a strong antioxidant activity and its concentration in the plasma is about 10 fold than antioxidants like vitamin C and vitamin E (200). , in the patient group , uric acid levels were not significantly different from those in the control group. We suggest that uric acid might not a strong antioxidant and might not protect against free radicals.

# SUMMARY

According to the results which obtained in this study we can show that although ROS would have an important role in the development of the oxidative damage in

both DM and RA, this peroxidative damage would occur through a complex mechanism with different factors involved in both diseases. The magnitude of this damage and its relationship with the antioxidant system were not the same in these pathologies. Also we can abbreviate the causes of no relation between both pathologies.

- Very high levels of MDA but normal UA were found in RA patients, this could indicate that the protective mechanism against oxidative damage would be independent of the antioxidant capacity of the plasma in both pathologies.
- The increase in the oxidation in DM patients would be related to the decrease of the antioxidant plasmatic capacity.

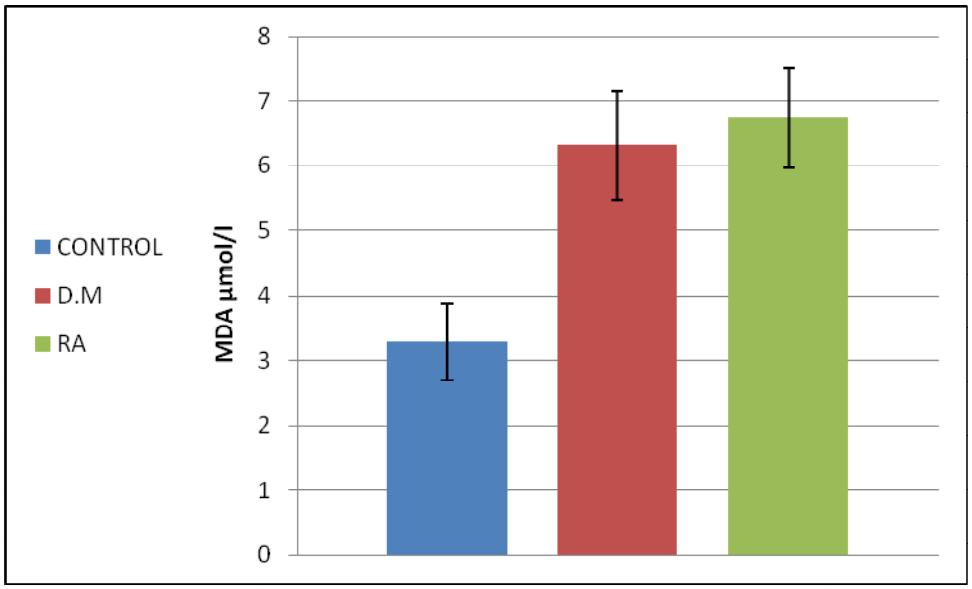


**Figure 1. Fig. 1 :The serum MDA levels in DM and RA samples compared with control samples**

**Table 1. Plasma MDA values in control, DM and RA patients**

| | Plasma MDA | | |
|---|---|---|---|
| | Control | DM | RA |
| Mean | 3.29 | 6.32 | 6.75 |
| ±SD | 0.59 | 0.84 | 0.77 |
| P-value | | significant | significant |

P-value statistical differences

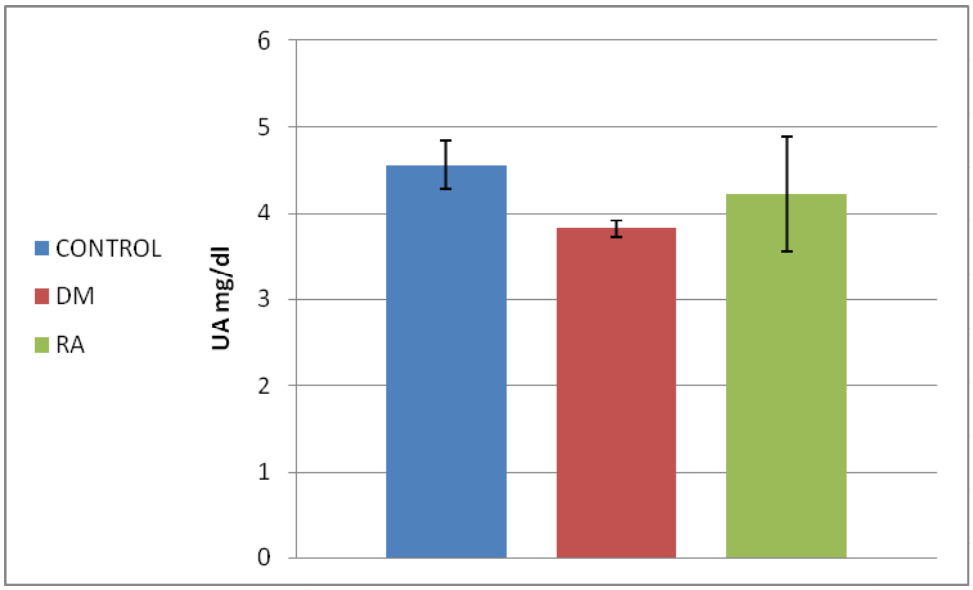


**Figure 2. Levels Fig. 1 :The serum UA levels in DM and RA samples compared with control samples**

**Table 2. Plasma MDA values in control, DM and RA patients**

| | plasma UA | | |
|---|---|---|---|
| | Control | DM | RA |
| Mean | 4.56 | 3.65 | 4.23 |
| ±SD | 0.29 | 0.11 | 0.66 |
| P-value | | significant | NS |

P-value statistical differences

# REFERENCES


1. Salem M. Kholoussi S.Kholoussi N.Fawzy R.Malondialdehyde and trace element levels in patients with type 2 diabetes mellitus.Archives of hellenic med. **2011**;28(1):83-88.
2. Nakhjavani M, Esteghamati A, Nowroozi S, Asgarani F, Rashidi A, Khalilzadeh O.Type 2 diabetes mellitus duration: an independent predictor of serum malondialdehyde levels.Singapore Med J **2010**; 51(7) : 582-585.
3. Slatter D.A. Bolton C.H. Bailey A.J.The importance of lipid-derived malondialdehyde in diabetes mellitus. Diabetologia **2000**; 43: 550-557.
4. SuryawanshiN.P. BhuteyA.K. NagdeoteA.N. JadhavA.A. ManoorkarG.S.study of lipid peroxide and lipid profile in diabetes mellitus. Indian J of ClinlBiochemy **2006**; 21 (1) :126-130.
5. Galli, F.; Piroddi, M.; Annetti, C.; Aisa, C.; Floridi, E.; Floridi, A. Oxidative stress and reactive oxygen species. *Contrib. Nephrol.*, **2005**;*149*: 240-260.

6. Gambhir, J. K.; Lali, P.; Jain, A. K. Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis.*Clin.Biochem.*,**1997**; *30*(4): 351-355.
7. Hitchon, C. A.; El Gabalawy, H. S. Oxidation in rheumatoid arthritis. *Arthritis Res. Ther.,* **2004**;*6*(6),:265-278.
8. Kamanli, A.; Naziroglu, M.; Aydilek, N.; Hacievliyagil, C. Plasma lipid peroxidation and antioxidant levels in patients with rheumatoid arthritis. *Cell Biochem.Funct.*,**2004**;*22*(1): 53-57.
9. Shaabani, Y.; Foroughi, M; Rastmanesh, R; Jamshidi, A; Tajik, N; Assadi, O. Assessment of antioxidant nutrient intake and Malondiadehyde plasma level in rheumatoid arthritis. *ARYA Atherosclerosis J*.**2009**; 5(1): 1-5.
10. Quinn L. Behavior and biology: The prevention of type 2 diabetes. J CardiovascNurs **2003**;18:62−68.
11. Mahreen R, Mohsin M, Nasreen Z, Siraj M, Ishaq M. Significantly increased levels of serum malonaldehyde in type 2 diabetics with myocardial infarction. *Int J Diabetes DevCtries* **2010**;30:49−51.
12. Panasenko, O. M.; Vol'nova, T. V.; Azizova, O. A.; Vladimirov, Y. A. Free radical modification of lipoproteins and cholesterol accumulation in cells upon atherosclerosis. *Free Radic. Biol. Med.,* **1991**,;*10*(2):137-148.
13. Scott DL, Symmons DP, Coulton BL, PopertAJ. Long-term outcome of treating rheumatoid arthritis: results after 20 years. Lancet **1987**;1:1108–11.
14. Mitchell DM, Spitz PW, Young DY, Bloch DA, McShane DJ, Fries JF. Survival, prognosis,and causes of death in rheumatoid arthritis.Arthritis Rheum**1986**;29:706–14.
15. Pincus T, Callahan LF, Sale WG, Brooks AL, Payne LE, Vaughn WK. Severe functional declines, work disability, and increased mortality in seventy-five rheumatoid arthritis patients studied over nine years. Arthritis Rheum **1984**;27:864–72.
16. Isomaki H. Long-term outcome of rheumatoid arthritis. Scand J Rheumatol Suppl **1992**;95:3–8.
17. Wolfe F. The natural history of rheumatoid arthritis. J Rheumatol Suppl **1996**;44:13–22
18. Paredes, S.; Girona, J.; Hurt-Camejo, E.; Vallve, J. C.; Olive, S.; Heras, M.; Benito, P.; Masana, L. Antioxidant vitamins and lipid peroxidation in patients with rheumatoid arthritis: Association with inflammatory markers. *J. Rheumatol*., **2002;** *29*(11):2271-2277.
19. Joan H. P. Robert B.Z. David A.L.Analysis of the thiol status of peripheral blood leukocytes in rheumatoid arthritis patients. J Leukocyte Biol.**2007**;81:1-8.
20. Satoh, K. Serum lipid peroxide in cerebrovascular disorder determined by new colorimetric method. Clin.Chim.Acta **1978**;90,:37-43.
21. WULF, D. Free radicals in the physiological control of cell function. Physiol Rev.**2002**;82: 47–95.
22. jain SK:Hyperglycemia can causes membrane lipid peroxidation and asmotic fragility in human red blood cells.J.Boil.Chem.**1989**;264:21340-21345.
23. Wierusz-Wysooka B,Wysocki H,Wyks H:Metabolic control aulity and free radicals activity in diabetic patients.Diabetetes res.Clin.Pract.**1995**;72. 193-207.

24. Nevi S,Bruno CM,Raciti C and Angelo B:altration of oxide reductive and harmostic factor in type 2diabetes J.Inter.Med.,**1994**;236(5):495-500.
25. Sarban, S.; Kocyigit, A.; Yazar, M.; Isikan, U. E. Plasma total antioxidant capacity, lipid peroxidation, and erythrocyte antioxidant enzyme activities in patients
26. Cotraneo P,Manto A,Todaro L,and Magnant p:Hyper filtration in patient with diabetes mellitus:a prevalence study:Clin Nephrol.**1998**;50:214-217.
27. Erdberg A,boner G,Van Dyck DJ and Care R:Uric acid excretion in patient with non-insulin dependent diabetes mellitus nephron,**1992**;60:134-137.
28. Gonzalez-Sicilia L,Garcia-Estan J,Martinez-Blazguez A and Frnandez-pardo J:Renal metabolism of uric acid in type 1 insulin dependent diabetic patient:Horm.Meta.Res.**1997**;29:520-523.
29. Sinclair AJ,Lunec,Girling AJ and Barnett AH:Modulatores of free radical activity in diabetes mellitus:Rol of ascorbic acid.EXS.1992;62:342-352.

# حالة التأثير التأكسدي للدهون(المالوندايالديهايد) وحامض اليوريك لمرضى السكري (النوع الثاني) ومرضى التهاب المفاصل الرثوي (دراسة مقارنة)

م.م. هبة عبد العظيم* د. احمد عبد الكاظم** د. مؤيد ناجي مجيد***

**الخلاصة:**

إن الهدف من هذه الدراسة للمقارنة الناتج التأكسدي (المالوندايالديهايد) وحامض اليوريك لمرضى الداء السكري (النوع الثاني) وفي أولئك المصابين بالتهاب المفاصل الرثوي. اجريت الدراسة على ٨٤ متبرع قسموا إلى مجموعتين لمرضى الداء السكري ومرضى التهاب المفاصل الرثوي ومتبرعين أصحاء. تم قياس مستوى الجهد التأكسدي (المالوندايالديهايد) وحامض اليوريك لهم.كان مستوى المالوندايالديهايد اقل في مجموعة السيطرة ( ٣،٢٩ ± ٠،٥٩ ميكرو مول- ديسليتر)على ما هو عليه في مرضى الداء السكري (النوع الثاني) ومرضى التهاب المفاصل الرثوي ( ٦،٣٢ ± ٠،٨٤ ميكرو مول- ديسليتر)، ( ٦،٧٤ ± ٠،٧٧ ميكرو مول- ديسليتر) على الترتيب عند ($p < 0.001$). علاوة على ذلك أثبتت الدراسة أن مستوى حامض اليوريك في مرضى السكري (النوع الثاني) ( ٣،٨١ ± ٠،١٠ ملغم- ليتر) هو اقل على ما هو عليه في مجموعة السيطرة ( ٤،٥٦ ± ٠،٢٨ ملغم- ليتر) وباحتمالية ($p < 0.001$)، بالمقابل لا يوجد تغير هام في مستوى المالوندايالديهايد في مرضى التهاب المفاصل ( ٤،٢٣ ± ٠،٦٦ ملغم - ليتر) مقارنة بالأصحاء.هذه النتائج اقترحت

* قسم الكيمياء الحياتية- كلية طب ذي قار
** قسم الفسلجة - كلية طب ذي قار
*** قسم طب الأطفال - كلية طب ذي قار