



## Immunodepressant and oxidant potential of standard leukaemia drug regimen

Marwan M Merkhan<sup>\*1</sup>, Ibrahim M Faisal<sup>2</sup>, Dema Z Alsaleem<sup>3</sup>, Omer M Shindala<sup>2</sup>, Hani M Almukhtar<sup>1</sup>, Imad A Thanoon<sup>2</sup>

<sup>1</sup>Department of Pharmacology and Toxicology, College of pharmacy, University of Mosul, Mosul-41002, Iraq

<sup>2</sup>Department of Pharmacology, College of Medicine, University of Mosul, Mosul-41002, Iraq

<sup>3</sup>Ninawa Health Department, Iraqi Ministry of Health, Mosul-41002, Iraq



### Article History:

Received on: 24 May 2020

Revised on: 25 Jun 2020

Accepted on: 26 Jun 2020

### Keywords:

AML,  
Cytotoxic,  
Immunoglobulin,  
Oxidative stress

### ABSTRACT

Chemotherapy improves cancer status but also could carry a risk of harmful side effects. In leukaemia patients, oxidative status and immune system are crucial parameters for patient improvement. The present study is aimed to assess the oxidative stress and the levels of immunoglobulin, including; IgG, IgA, and IgM in acute myelocytic leukaemias (AML) patients. A total of twenty-one patients with AML were enrolled in this study, alongside 24 healthy individuals as a control group. Initially, in both groups, the serum MDA, IgA, IgG, and IgM levels were measured. Then after a month of treatment with standard leukaemia therapy; the measurement of levels of these parameters were repeated for both the patients' group and the control group for measuring immunoglobulin levels while analysis of MDA was done by the laboratory method. A highly significant rise in the serum MDA level was detected ( $P < 0.001$ ) in patients with AML after treatment with specific cytotoxic drugs was observed in comparison with the control. Moreover, there was a significant modulation in the serum concentration of Ig after starting cytotoxic drugs in comparison with the controls. The study revealed that AML was associated with elevated oxidative stress parameters following treatment with cytotoxic drugs as reflected by the rise in the serum levels of MDA. The decreased serum Ig levels indicate a reduction in immune response in patients with AML due to cytotoxic drugs.

### \*Corresponding Author

Name: Marwan M Merkhan

Phone: 009647508662343

Email: [marwanmerkhan@uomosul.edu.iq](mailto:marwanmerkhan@uomosul.edu.iq)

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i4.3199>

Production and Hosted by

IJRPS | [www.ijrps.com](http://www.ijrps.com)

© 2020 | All rights reserved.

### INTRODUCTION

Intensive chemotherapy is the standard of cancer management, and recent studies suggested that cancer were associated with the reduction of constitutive pools of antioxidants, while others showed that chemotherapy exposure results in a higher level of oxidative stress than cancer by itself (Conklin, 2004; Papageorgiou *et al.*, 2005). Reactive oxygen species (ROS) plays a crucial role in normal biological and physiological functions; including, cell signalling, the activity of phagocytes and are involved in a mitochondrial redox reaction, they may result in an oxidative injury of cellular, subcellular, and extracellular components and have been involved in

the prognosis of various diseases mainly the chronic ailments (Papageorgiou *et al.*, 2005). Free radicals and oxidative stress reaction have been involved in the pathophysiology of leukaemia (Singh *et al.*, 2001). Antioxidants are free radical scavengers and hence protect aerobic organisms from the oxidative stress of typical daily metabolic activity by normalizing the damaging effect of free radicals of usual daily metabolic activity within the body. Moreover, antioxidants protect the important functional biomolecules (carbohydrate, fat, protein, and DNA) from oxidative damage (Kerr *et al.*, 1996).

In addition to the role of antioxidants in protecting against acute oxidative stress, they also reflect their consumption during such stress conditions. Tissue injury result from the imbalance between oxidant-antioxidant status (Young and Woodside, 2001).

Our study aimed to confirm the adverse effects of currently in-use standard leukaemia regimen on oxidative stress and the concentration of some immunological parameters, including IgA, IgG, and IgM in patients with acute myelocytic leukaemia (AML) before and after standard therapy.

## PATIENTS AND METHODS

This study was conducted in the Nuclear Medicine Hospital in Mosul City. Patients diagnosed with AML were interviewed and only those matched the study criteria of being diagnosed with AML and planned to be treated with specific cytotoxic drugs, having no other diseases and non-smoker were included. The specific cytotoxic regimen used were a combination of doxorubicin (dose, 25mg/m<sup>2</sup>; duration, three days) and cytosine arabinoside (dose, 100mg/m<sup>2</sup>; duration, seven days).

Thirty-one cases were interviewed, only 24 satisfied the inclusion criteria of selection and included in the present research. One case died, and another 2 cases need the addition of Etoposide (dose, 100mg/m<sup>2</sup>; duration 3 days) to their regimen, and these cases were excluded from the study. Finally, 21 cases completed the study (16 males/5 females; age 49.10±9.06 year). A 24 healthy, non-smoker subjects, were enrolled in the study as a control group (19 males/5 females; age 49.54±8.73 year).

Initially, 7 ml of venous blood samples were taken for the assay of serum MDA and immunological parameters, including IgA, IgG, and IgM; from both groups. After a period of one-month for the control and one-month from starting the specific cytotoxic drugs for the patients, another 7ml venous blood samples were taken for the assay of the mentioned parameters.

The measurements of MDA in the serum was done according to the method of Buege and Aust (1978). The immunoglobulin levels were measured using the immunoturbidimetric method with standard kits (Randox Company, UK).

The results of the normally distributed variables were represented as mean±SD. To compare the measured parameters between studied groups, Mann-Whitney test was used. The differences were considered significant when p<0.05. All statistical results were conducted using GraphPad Prism 6 (CA, USA). The histograms were drawn using a Microsoft Office 2010 Excel program.

## RESULTS AND DISCUSSION

### Immunodepressant potential of standard leukaemia drug regimen

The level of different immunoglobulins (Ig) was measured including, IgG, IgA, and IgM in control healthy subjects and these levels were compared to patients before and after exposure to standard leukaemia therapeutic regimen. The results reveal that IgM showed no significant changes in their levels in control healthy group as compared to their levels in the patient group weather before or after therapy (p>0.05). However, IgG shows a non-significant reduction in leukemic patients as compared to control healthy subjects (p>0.05) and a further significant decrease (p<0.001) were achieved with the initiation of the therapy. Moreover, IgA showed a substantial reduction in patients compared to healthy control subjects with further reduction achieved with the start of the therapy (p<0.001). The details of these changes are outlined in Figure 1.

One-way ANOVA conducted to indicate the differences between group. Each column of the histogram represents mean±SD (n=20). #p<0.05 and ##p<0.001 comparison in patients' group before and after therapy, \*p<0.05, \*\*p<0.001, and \*\*\*p<0.0001 as compared to healthy control group. CHG=control healthy group, BTG=before therapy group, ATG=after therapy group (Figure 1).

### Oxidant potential of standard leukaemia drug regimen

The level of malondialdehyde (MDA) was measured in control of healthy subjects, and these levels were compared to patients before and after exposure to standard leukaemia therapeutic regimen. The results reveal that MDA showed a significant elevation in their levels in the patients' group as compared to control healthy group with further elevation achieved with the initiation of the therapy (p<0.001). The details of these changes are outlined

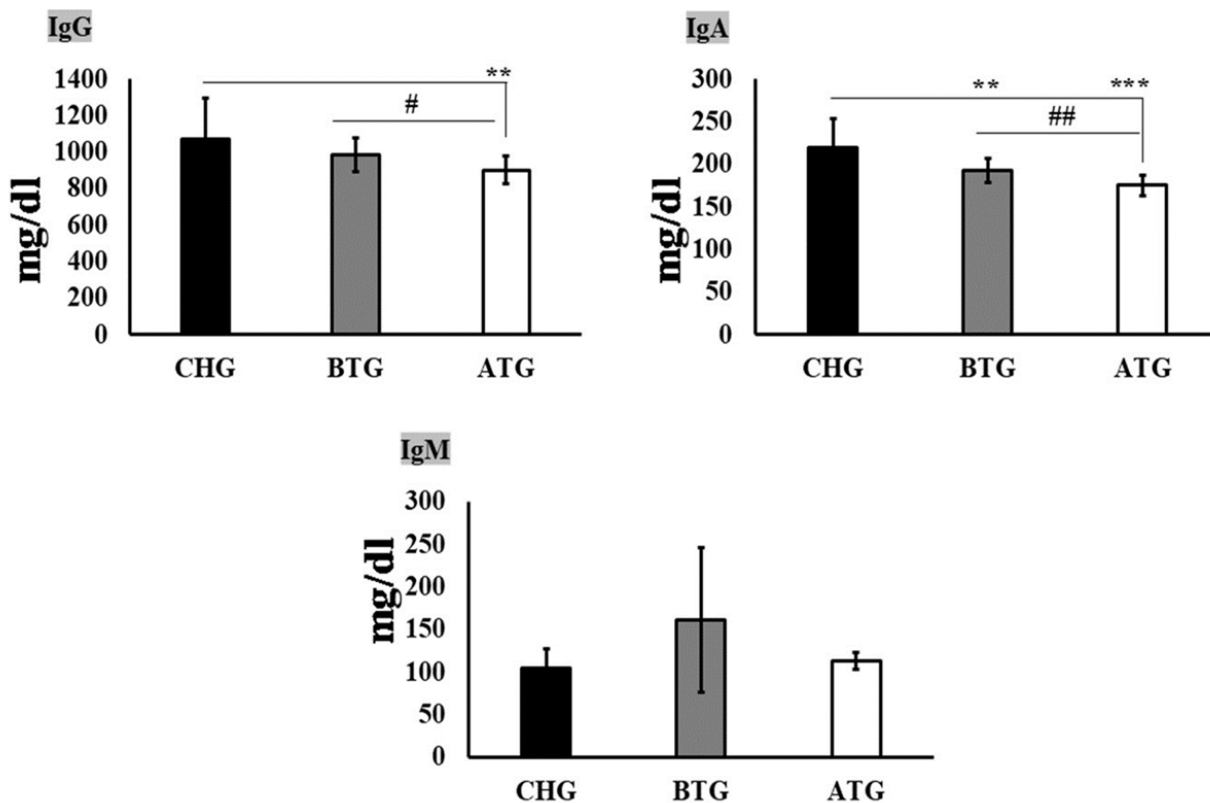


Figure 1: Consecutive determinations of serum IgG, IgA, and IgM in patients with Acute Myelocytic Leukaemia under chemotherapy

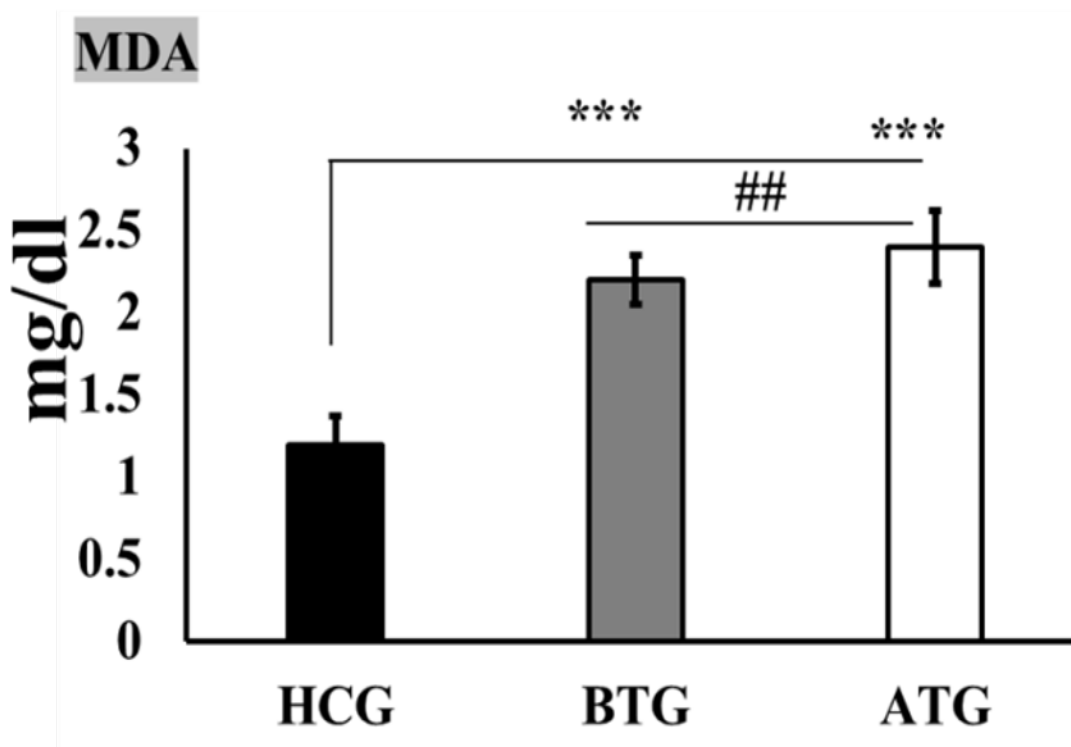


Figure 2: Oxidative stress in patients with Acute Myelocytic Leukaemia under chemotherapy indicated by malondialdehyde (MDA) measurement

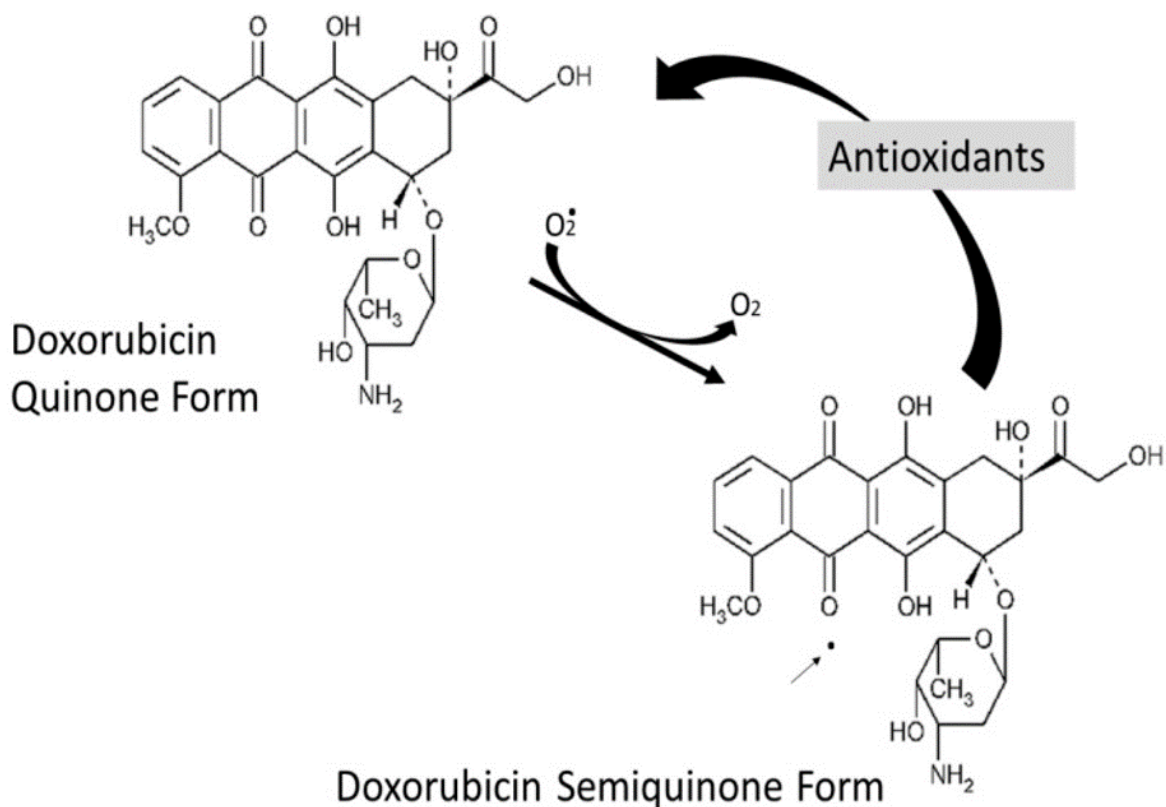


Figure 3: Redox system based on Chemical formula of doxorubicin and associated enzymatic oxidation/reduction cycle

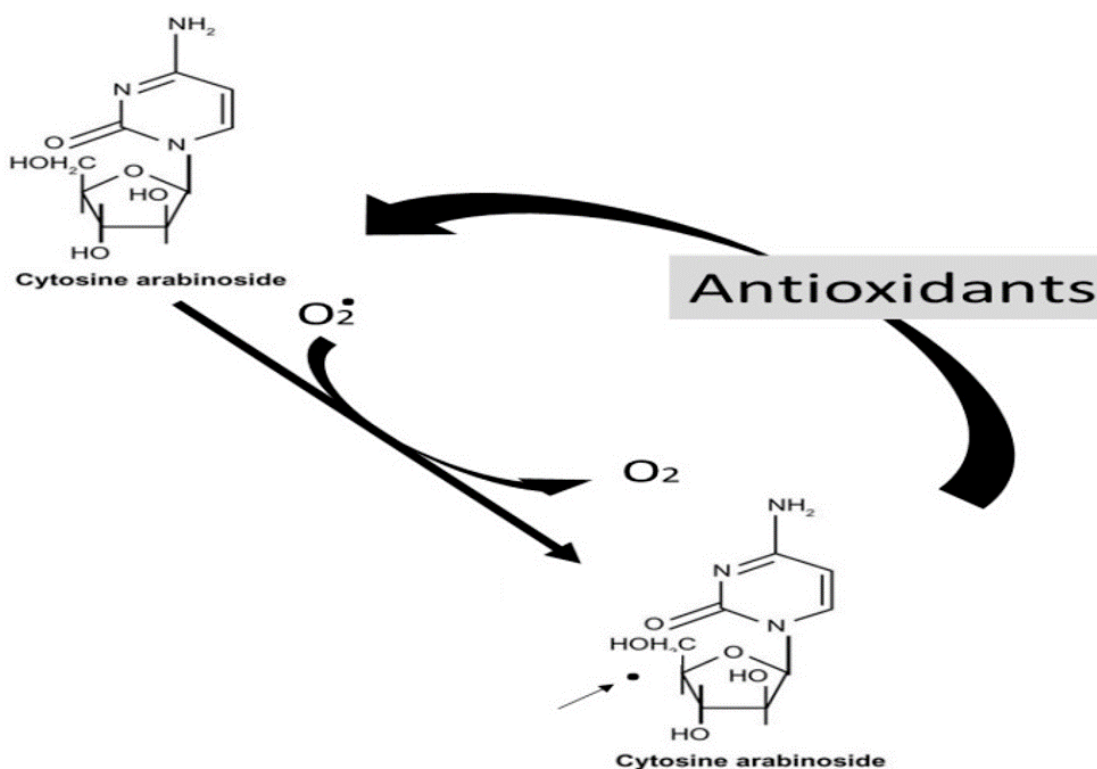


Figure 4: Redox system based on Chemical formula of cytosine arabinoside and associated enzymatic oxidation/reduction cycle

in Figure 2.

Each column of histogram represent mean $\pm$ SD (n=20). ##p<0.001 comparison in patients' group before and after therapy, \*\*\*p<0.0001 as compared to control healthy group. CHG=control healthy group, BTG=before therapy group, ATG=after therapy group (Figure 2).

Our results confirmed a significant increase in serum MDA concentration (p<0.001) in patients with AML after 1 month from starting specific cytotoxic drugs in comparison to the control. The immunological results demonstrated that cancer patients have altered levels of immunoglobulins (significantly lower IgA and IgG and non-significantly higher IgM) compared to controlled healthy subjects and anticancer significantly reduced IgA and IgG level with no changes observed with IgM levels.

Lipid peroxidation is a series of reactions providing a continuous supply of free radicals (Mayes, 2000). The MDA is most widely used parameter in clinical laboratories to monitor the oxidative stress and as an index of lipid peroxidation (Draper and M, 1990). Leukemia is of unknown causative agent and free radicals have been shown to be part of the aetiology of leukemia. The chemical structure of MDA closely resembles to that of glyceraldehyde and beta-propiolactone both are known to be carcinogens (Singh et al., 2001).

Ghalaut et al. (1999) observed a significant increase in MDA serum levels in active phase of acute as well as in chronic leukaemia than those in remission states. Moreover, Singh et al. (2001) also reported a rise in the serum MDA concentration in acute lymphocytic leukaemia (ALL) patients which were higher in active phase of the disease than in the remission phase.

A study conducted by Bakan et al. (2003) confirmed that MDA concentration is a representative marker for lipid peroxidation, referring to the enzymatic antioxidant activity (serum activity of glutathione peroxidase with glutathione reductase) confirming that there are significant modulation of oxidant/antioxidant systems in patients with chronic lymphocytic leukemias.

Pediatric studies, conducted in different countries, involving determination of the oxidant/antioxidant balance in malignant children has demonstrated that the levels of intrinsic antioxidant pools were dramatically lower than their counterpart healthy normal subject confirmed by measurement of antioxidant enzymatic activity, vitamin C, vitamin E, total antioxidant status, and serum MDA

levels (Sentürker et al., 1997; Malvy et al., 1997).

The processing of neoplasm and associated growing of cancer cell division makes the cancer cells to utilize more antioxidant vitamins than normally dividing controlled cells, this conflicting behaviour of cancer cells were believed to exhaust the host serum antioxidant biomolecules. These contradictory theories have been confirmed by measuring the antioxidant biomolecules before and after anti-cancer therapy and their comparisons in a patient with different types of neoplasm confirming the above hypothesis (Ray et al., 2000; Agus et al., 1999). However, the situation might be more challenging after starting the treatment with anticancer drugs because chemotherapy by itself might affect antioxidant-dietary consumption. Moreover, the prognosis of cancer following treatment might be potentially associated with better serum antioxidant activity; the outcomes were more linked to the specific agents, type of neoplasm, the stage of the disease (Durken et al., 1995; Dürken et al., 2000). That is why, in this study, we select patients on the same cytotoxic agents (Figure 3 and Figure 4).

A study conducted by Gadjeva et al. (2005) on patients with lymphoproliferative haematological diseases; confirmed an imbalance between oxidant-antioxidant biomolecules as confirmed by measurement of serum MDA level and a reduction in serum enzymatic activity of superoxide dismutase as a marker of antioxidant activity. The study has concluded that a combination of chemotherapy used concurrently associated with higher oxidative stress and lower antioxidant activity (Gadjeva et al., 2005).

With Igs level in patients with leukaemia, only a single published work by Solanki et al. (1990) found that there is a typical concentration or even decreased serum immunoglobulins in different types of lymphoma and leukaemia patients and at various stages of disease progression, the limitation of this study is the small number of patients with AML, which was only five (Solanki et al., 1990; Enad and Wa, 2019).

This study which involved 19 AML patients revealed a highly significant difference in the serum concentration of immunoglobulins (IgA, IgG, IgM) before and after chemotherapy and in comparison, with controls (p<0.001).

## CONCLUSION

In conclusion, AML disease is associated with the impaired balance between oxidative/antioxidant system during cytotoxic regimen as reflected by a rise in serum MDA which might be potentially



reduced following addition of antioxidant (e.g. vitamin E and C) therapy to patients with AML during cytotoxic therapy. Such recommendation necessitates further controlled laboratory studies on rat or mice using individual anticancer drugs and combinations to answer such a question. The explanation behind reduced immunoglobulins level in patients with AML during cytotoxic therapy might be due to the reduced immunity of such patients during such period. The immunodepressant could be transient, i.e. associated with the regimen administration, necessitating continuous follow up to confirm the normality of the level during dosing intervals.

### ACKNOWLEDGEMENT

The researcher is thankful to the University of Mosul for their provided facilities, which helped to improve the quality of this work. Thanks are also for the scientific committee and ethics committee in the college of medicine-university of Mosul.

### Conflict of interest

The authors declare that they have no conflict of interest for this study.

### Funding support

The authors declare that they have no funding support for this study.

### REFERENCES

- Agus, D. B., Vera, J. C., Golde, D. W. 1999. Stromal cell oxidation : A mechanism by which tumours obtain vitamin C. *Cancer Res*, 59:4555–4558.
- Bakan, N., Taysi, S., Yilmaz, Ö., Bakan, E., Kuşay, S., Uzun, N., Gündoğdu, M. 2003. Glutathione peroxidase, glutathione reductase, Cu-Zn superoxide dismutase activities, glutathione, nitric oxide, and malondialdehyde concentrations in serum of patients with chronic lymphocytic leukemia. *Clinica Chimica Acta*, 338(1-2):143–149.
- Buege, J. A., Aust, S. D. 1978. Methods in enzymology. Biomembranes part C: Biological oxidations, microsomal cytochrome p-450 and other hemo-protein systems. In Fleischer, S., Packer, L., editors, *Methods in enzymology*, San Francisco. Elsevier. Pages 302 - 310.
- Conklin, K. A. 2004. Cancer Chemotherapy and Antioxidants. *The Journal of Nutrition*, 134(11):3201S–3204S.
- Draper, H. H., M, H. 1990. Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology*, 186:421–431.
- Durken, M., Agbenu, J., Finckh, B., Hubner, C., Pichlmeier, U., Zeller, W. 1995. Deteriorating free radical-trapping capacity and antioxidant status in plasma during bone marrow transplantation. *Bone Marrow Transplantation*, 15(5):757–762.
- Dürken, M., Herrnring, C., Finckh, B., Nagel, S., Nielsen, P., Fischer, R., Berger, H. M., Moison, R. M., Pichlmeier, U., Kohlschütter, B., Zander, A. R., Kohlschütter, A. 2000. Impaired plasma antioxidative defense and increased nontransferrin-bound iron during high-dose chemotherapy and radiochemotherapy preceding bone marrow transplantation. *Free Radical Biology and Medicine*, 28(6):887–894.
- Enad, S. A., Wa, A.-A. 2019. Investigation of Secondary Acute Lymphoblastic Leukemia (sALL) Among Acute Lymphoblastic Leukemia (ALL) Iraqi Patients. *Iraqi Journal of Science*, 60(2):223–227.
- Gadjeva, V., Kuchukova, D., Georgieva, R. 2005. Influence of polychemotherapy on the antioxidant levels and lipid peroxidation in patients with lymphoproliferative diseases. *Comparative Clinical Pathology*, 14(1):13–18.
- Ghalaut, V. S., Ghalaut, P. S., Singh, S. 1999. Lipid peroxidation in leukaemia. *The Journal of the Association of Physicians of India*, 47(4):403–405.
- Kerr, M. E., Bender, C. M., Monti, E. J. 1996. An introduction to oxygen free radicals. *Heart and Lung*, 25(3):200–209.
- Malvy, D. J.-M., Arnaud, J., Burtschy, B., Sommelet, D., Leverger, G., Dostalova, L., Amédée-Manesme, O. 1997. Antioxidant micronutrients and childhood malignancy during oncological treatment. *Medical and Pediatric Oncology*, 29(3):213–217.
- Mayes, P. A. 2000. Structure and function of lipid soluble vitamins and the pentose phosphate pathway and other pathways of hexose metabolism. In Murray, R. K., Granner, D. K., Mays, P. A., Rodwell, V. W., editors, *Harpers Biochemistry*. McGraw-Hill. 25th Edition.
- Papageorgiou, M., Stiakaki, E., Dimitriou, H., Malliaraki, N., Notas, G., Castanas, E., Kalmanti, M. 2005. Cancer chemotherapy reduces plasma total antioxidant capacity in children with malignancies. *Leukemia Research*, 29(1):11–16.
- Ray, G., Batra, S., Shukla, N. K., Deo, S., Raina, V., Ashok, S., Husain, S. A. 2000. Lipid peroxidation, free radical production and antioxidant status in breast cancer. *Breast Cancer Research and Treatment*, 59(2):163–170.
- Sentürker, S., Karahalil, B., Inal, M., Yilmaz, H., Müslümanoğlu, H., Gedikoglu, G., Dizdaroglu, M. 1997. Oxidative DNA base damage and antioxidant enzyme levels in childhood acute lymphoblastic

- leukemia. *FEBS Letters*, 416(3):286–290.
- Singh, V., Ghalaut, P. S., Kharb, S., Singh, G. P. 2001. Plasma concentration of lipid peroxidation products in children with acute leukaemia. *Indian Journal of Medical Science*, 55(4):215–222.
- Solanki, R. L., Anand, V. K., Arora, H. L. 1990. Serum immunoglobulins in leukaemia and malignant lymphoma. *Journal of the Indian Medical Association*, 88(11):305–307.
- Young, I. S., Woodside, J. V. 2001. Antioxidants in health and disease. *Journal of Clinical Pathology*, 54(3):176–186.