

Original Research Article

Ischemia Modified Albumin Level in Patients with Iron Deficiency Anemia

Abstract

Background: Anaemia happens when s'the blood level of red blood cells (RBCs) and/or haemoglobin are lower than normal for age and sex. The most prevalent type of anaemia is (IDA)iron deficiency anemia . Timshis study a to measure the level of ischemia modified albumin (IMA) in patients with IDA prior to both and owingfoll iron supplementation therapy and to spotlight on its correlation with severity of anemia and to assess clinical outcomes after iron therapy with respect to oxidative stress.

Methods: This study was carried out on 30 iron deficiency anaemia patients and 20 healthy controls with matched age and sex. Three groups were formed out from the patients, Group I: Twenty healthy subjects as a control with no history of IDA, group II: Thirty patients IDA before iron treatment and group III: The same thirty patients with IDA, but after oral iron treatment (100 mg ferrous glisin sulfate twice daily for two months). All patients were subjected to routine laboratory investigation [Hb, Htc, MCV, Fe, TIBC, ferritin, transferrin saturation, reticulocytic count and albumin], and specific laboratory investigation (ischemia modified albumin).

Results: In group II, there was positive significant correlation between MCV and IMAR, there was positive significant correlation between IMA level and MCV, while there was negative significant correlation between Hb, haematocrit, albumin, and IMA. In group III, there was negative significant correlation between albumin and IMAR, there was negative significant correlation between Hb, Htc, albumin, and IMA.

Conclusions: Anemia causes a rise in IMA, and this increase may be related to hypoxia. And we found that the IMA levels could be demonstrative of the severity of anemia. Its level was

elevated after iron therapy (100 mg ferrous gluconate sulphate twice daily for two months) attributed to oxidative stress associated with iron therapy, which might alter the structure of albumin and raise IMA values.

Keywords: Ischemia Modified Albumin, Iron Deficiency Anemia, Iron Supplementation, Clinical Outcomes

Introduction:

Anaemia ensues when the level of red blood cells (RBCs) and/or haemoglobin are lower than normal for age and sex. The most prevalent type of anaemia is iron deficiency anemia (IDA).

Iron is necessary for the human body to make hemoglobin, which carries oxygen to body tissues and is necessary for the muscles and tissues for optimal function.^[1]

There are between 1 and 2 billion persons who suffer from iron deficiency anaemia worldwide. Over 50% of women who are pregnant in developing nations are anaemic, and iron deficiency is thought to be the cause of 46–66% of anaemia in children under the age of 4.^[2]

Albumin of the human serum consists of a single chain of 585 amino acids, made up of three homologous structures, large helical domains (I, II, and III).

A and B are the two subdomains that make up each domain. Asp-Ala-His, the first 3 amino acids in the albumin's N-terminus, act as a particular site of binding for transition metals including nickel (II), copper (II), and cobalt (II), and are also the region that is most prone to degradation comparing with the other albumin regions.^[3]

An oxidative stress marker called ischemia-modified albumin (IMA) rises when there is acidosis, hypoxia, or damage of free radicals.^[4]

Prior studies have shown that serum IMA levels rise in a number of ischemic conditions, such as IDA, pulmonary embolism, acute coronary syndrome, ischemia of the lower extremities, and central nervous system diseases associated with ischemia.

The increase in IMA in IDA patients is caused by hypoxia as hypoxia is responsible for modification in N-terminus of serum albumin. In addition to oxidative stress occurs with oral iron that could increase IMA^[4].

This study aims to measure the level IMA in patients with IDA prior to both and following iron supplementation therapy and to spot light on its correlation with severity of anaemia and to assess clinical outcomes after iron therapy with respect to oxidative stress.

Materials and Methods:

This research included 30 iron deficiency anaemia patients more than 18 years, newly diagnosed, menstruating women and they didn't receive any treatment and 20 healthy controls with matched age and sex were taken from the Tanta University Hospitals' Clinic of Internal Medicine Department (from June 2017 to December 2017).

Exclusion criteria were patient with malnutrition, hepatitis, diabetes obesity, cancer, alcoholism, smoking, viral, other ischemic diseases, other severe diseases, congenital disorders and under therapy.

Three groups were formed out from the patients: Group I: Twenty healthy subjects as a control with no history of IDA, group II: Thirty patients with IDA before iron treatment and group III: The same thirty patients with IDA, but after oral iron treatment (100 mg ferrous glisin sulphate twice daily for two months).

All participants had thorough taken history, examined clinically, and routine lab investigations [Hb, Htc, MCV, Fe, TIBC, ferritin, transferrin saturation, reticulocytic count and albumin], and specific laboratory investigation (ischemia modified albumin).

Iron

Guanidine hydrochloride releases ferric ions from their transfer and hydroxylamine reduces them to their ferrous state. A coloured complex is created when ferrous ions and ferrozine interact. To prevent copper interference, cupric ions are bound to thiourea. By monitoring the rise in absorption at 546 nm, the colour intensity is calculated to be in direct proportion to the concentration of the iron.

By exposing the specimen to excessive ferric ions, the transferrin becomes saturated with iron. Unbound iron is subsequently eliminated by centrifugation and the application of light magnesium carbonate. In the supernatant, the amount of iron bonded to protein is estimated.

Mean corpuscular volume (MCV) and transferrin saturation (TS) were calculated by Lee and Nieman, (1996) according to the following equations:

$$MCV = \frac{Htc \times 10}{RBCs}$$

Ferritin ^[5]:

Method: Enzyme-linked immunoassay kits, accessible from Immune Spec Cooperation at 7018 Owens Mouth Avenue, suit *103 Cango Bank, CA 91303, were used to assess serum ferritin.

Laboratory assessment of IMA by enzyme-linked immune- sorbent assay (ELISA) ^[6]:

IMA was estimated by Enzyme linked immunosorbent assay (ELISA) kit supplied by SunRed Biology Company. ■ Reagent provided: 1. Micro – ELISA strip plate: 12 well x 8 strips, 2.

A typical vial holds 0.5 ml. 3. standard diluent (SD) comprises 3 ml. 4. A vial of the conjugate reagent streptavidin-HRP (horseradish peroxidase) contains 6 ml. 5. One vial of the wash solution (30 x concentration) contains 20 ml. 6. One vial of biotin-IMA-ag ab contains one millilitre. 7. One vial of chromogen solution A contains 6 ml. 8. One vial of chromogen solution B contains 6ml. 9. Stop solution: Each vial has 6ml in it.

Standard dilution: 320ng/ml Standard No (SN).5 120µl original standard (OS)+120µl SD
160ng/ml SN.4 120µl SN.5+120µl SD 80ng/ml SN.3 120µl SN.4+120µl SD
40ng/ml SN.2 120µl SN.3+120µl SD 20ng/ml SN.1 120µl SN.2+120µl SD.

Blank wells: IMA-Ag-antibodies labelled with biotin, samples, and streptavidin-HRP were not administered, instead, only chromogen solutions A and B and stop solution were enabled. Standard wells were added 50 µl of streptavidin-HRP to the standard 50 µl (It was unnecessary to add antibody because the standard already contains combined biotin antibody). Test wells: 40 µl of specimen were added, followed by 50 µl of streptavidin-HRP and 10 µl of the IMA -Ag antibody. The sealing membrane was then sealed, shaken gently, and incubated at 37 °C for 60 minutes.

Confection: Distilled water was used as a reserve to dilute the washing solution (20 x concentration) 20 times. Washing: After gently removing the membrane, draining the liquid, and shaking away any residual water. Each well received 50 µl each of chromogen solutions A and B before being well blended and incubated for 10 mins at 37 °C away from light.

50 µl of the stop solution were added to each well to finish the reactions (The blue colour was immediately replaced with yellow).

The optical density (OD) was measured at 450 nm wavelength as the final measurement, which should be done within 15 minutes after applying the stop solution. Blank wells were used as the baseline.

The OD values of the samples were determined using the regression equation to determine the corresponding sample's concentration based on the standard's concentration and the corresponding OD values.

Statistical analysis

Statistical analysis was done by SPSS V.22. Shapiro-Wilks test and histograms were used to evaluate the normality of the distribution of data. Quantitative parametric data were presented

as mean and standard deviation (SD) and were analysed by ANOVA (F) test with post hoc test (Tukey). Qualitative variables were presented as frequency and percentage (%) and were analysed utilizing the Chi-square test. Linear Correlation coefficient (r) was used for detection of correlation between two quantitative variables in one group. A two tailed P value < 0.05 was considered statistically significant.

Results:

There were no statistically substantial changes of albumin levels among the studied groups (P = 0.423). There was a statistically marked rise in serum IMA, IMAR, levels of TIBC, Transferrin saturation, and reticulocyte count, among the studied groups (P = 0.001). There was a statistically marked educer in Fe, ferritin, haematocrit and MCV levels the groups undergoing study (P = 0.001).

Table 1: Hb level, Htc, MCV, Serum ferritin level, serum iron TIBC, albumin level, IMA, IMAR, reticulocyte count and Transferrin saturation in the studied groups.

		Mean± S. D	p. value		
Hb (g/dl)	Group I	13.67±0.89	0.001*	P1	0.001*
	Group II	9.93±1.07		P2	0.011*
	Group III	12.96±0.43		P3	0.001*
Htc (%)	Group I	40.89±1.88	0.001*	P1	0.001*
	Group II	32.22±2.64		P2	0.001*
	Group III	37.73±2.68		P3	0.001*
MCV (fl)	Group I	88.23±2.50	0.001*	P1	0.001*
	Group II	74.55±3.26		P2	0.001*
	Group III	81.63±3.83		P3	0.001*
Ferritin (ng/ml)	Group I	55.03 ± 19.12	0.001*	P1	0.001*
	Group II	7.23 ± 2.38		P2	0.001*
	Group III	14.50 ± 1.27		P3	0.044*
Serum iron (ug/dl)	Group I	95.71 ± 17.56	0.001*	P1	0.001*
	Group II	29.01 ± 16.56		P2	0.004*
	Group III	80.72 ± 13.36		P3	0.001*
TIBC (ug/dl)	Group I	317.26 ± 31.23	0.001*	P1	0.001*
	Group II	410.23 ± 23.40		P2	0.001*
	Group III	365.18 ± 20.28		P3	0.001*
Reticulocyte (%)	Group I	1.51± 0.51	0.001*	P1	0.191
	Group II	1.33± 0.46		P2	0.001*
	Group III	2.90± 0.28		P3	0.001*
Transferrin saturation (%)	Group I	35.85± 8.81	0.001*	P1	0.001*
	Group II	14.15 ± 2.25		P2	0.020*
	Group III	40.35 ± 4.88		P3	0.001*

Albumin (g/dl)	Group I	4.32±0.29	0.423	P1	0.802
	Group II	4.29±0.35		P2	0.216
	Group III	4.19±0.34		P3	0.323
IMA (ng/ml)	Group I	247.08±59.56	0.001*	P1	0.001*
	Group II	433.30±83.17		P2	0.001*
	Group III	612.05±22.57		P3	0.001*
IMAR (IMA/Al)	Group I	54.95±12.97	0.001*	P1	0.001*
	Group II	100.80±16.47		P2	0.001*
	Group III	146.91±9.68		P3	0.001*

Hb: hemoglobin, Htc: Hematocrit, MCV: Mean corpuscular volume, TIBC: Total iron binding capacity, IMA: Ischemia modified albumin, *: significant P value

There was positive significant correlation between IMA level and MCV, while there was negative significant correlation between Hb, haematocrit, albumin and IMA. While there was no correlation between ferritin, iron, TIBC, reticulocytic count, transferrin saturation and IMA.

Table 2: Correlation between IMA level and studied parameters in group II

Group II	IMA	
	r	P
Hb	- 0.665	0.003*
Htc	-0.607	0.005*
MCV	0.578	0.008*
Ferritin	-0.124	0.602
Albumin	-0.486	0.042*
Iron	-0.326	0.161
TIBC	-0.049	0.839
Reticulocyte (%)	0.156	0.512
Transferrin sat (%)	0.286	0.221

Hb: hemoglobin, Htc: Hematocrit, MCV: Mean corpuscular volume, TIBC: Total iron binding capacity, IMA: Ischemia modified albumin, *: significant P value

There was positive significant correlation between MCV and IMAR level while there was no correlation between Hb, Htc, ferritin, TIBC, albumin, reticulocytic count, transferrin saturation and IMAR.

Table 3: Correlation between IMAR level and studied parameters in a group II

Group II	IMAR	
	r	p
Hb	0.356	0.123
Htc	-0.131	0.582
MCV	0.617	0.004*
Ferritin	-0.093	0.698

Albumin	0.103	0.664
Iron	-0.371	0.108
TIBC	-0.041	0.864
Reticulocyte (%)	0.174	0.462
Transferrin saturation (%)	0.313	0.179

Hb: hemoglobin, Htc: Hematocrit, MCV: Mean corpuscular volume, TIBC: Total iron binding capacity, IMA: Ischemia modified albumin, *: significant P value

There was negative significant correlation between Hb, Htc, albumin and IMA, while there was no correlation between MCV, ferritin, iron, TIBC, reticulocytic count, transferrin saturation and IMA.

Table 4: Correlation between IMA level and studied parameters in group III.

Group III	IMA	
	r	P
Hb	-0.764	0.001*
Htc	-0.529	0.016*
MCV	-0.089	0.708
Ferritin	-0.349	0.132
Albumin	-0.506	0.038*
Iron	-0.297	0.204
TIBC	0.004	0.987
Reticulocyte (%)	0.156	0.510
Transferrin saturation (%)	0.198	0.402

Hb: hemoglobin, Htc: Hematocrit, MCV: Mean corpuscular volume, TIBC: Total iron binding capacity, IMA: Ischemia modified albumin, *: significant P value

There was negative significant correlation between albumin and IMAR, while there was no correlation between Hb, Htc, MCV, ferritin, iron, TIBC, reticulocytic count, transferrin saturation and IMAR.

Table 5: Correlation between IMAR and studied parameters in group III.

Group III	IMAR	
	r	p
Hb	-0.159	0.502
Htc	0.181	0.444
MCV	-0.010	0.966
Ferritin	-0.145	0.541
Albumin	-0.873	0.001*
Iron	-0.004	0.987
TIBC	0.229	0.332
Reticulocyte (%)	0.009	0.971
Transferrin saturation (%)	0.142	0.550

Hb: hemoglobin, Htc: Hematocrit, MCV: Mean corpuscular volume, TIBC: Total iron binding capacity, IMA: Ischemia modified albumin, *: significant P value

Discussion

IDA is a widespread health issue that affects people of all ages. It is a frequent source of morbidity and is responsible for 50% of anaemia cases globally. Pregnancy, adolescents, and the initial two years of life are when it occurs most frequently. Pallor, fatigue, koilonychia and weakness are its characteristics. Dietary modifications and intravenous and oral iron supplementation are the mainstays of treatment.^[7]

In the present work, there was statically significant decrease in different CBC parameters (Hb, Mcv and hematocrit) in group II in comparison to group I and group III because the iron is transported by transferring to the bone marrow for synthesis of hemoglobin and incorporated into the erythrocytes and in IDA patients there is decreased iron level. The same outcomes were reported by Turk et al.,^[8] who found that cases with iron deficiency anemia showed statically significant decrease in different CBC parameters (Hb, hematocrit, MCV, MCH and RBCs) in comparison to cases with normal iron state. In group III, Hb, MCV and Htc results raised after therapy.

Furthermore, serum ferritin levels decreased statically significantly in the group II in comparison to group III and group I because our bodies' ability to store iron is aided by the protein ferritin. owing to inadequate iron storage, ferritin levels are low. similar results were reported by Mansour Babaei.,^[9] who found that, IDA patients had considerably lower mean serum ferritin levels than those who did not have IDA.

As regard TIBC and Transferrin saturation, there was statically significant decrease transferrin saturation in group II in comparison to group I and group III, because Transferrin is a protein that transports iron and in IDA the level of iron is decreased. but TIBC increased statically significantly in group II in comparison to group III and group I, because the quantity of iron carried by transferrin and the level of iron are assessed using the TIBC test, this is consistent with van Santen et al.,^[10].

As regard iron level, there was decrease in its level in group II in comparison to group III and group I, that is due to in patients with IDA, there is decreased iron intake, impaired iron absorption, hemorrhage and bleeding but after treatment iron storage sites will be saturated and the level of free iron in the blood will be increased comparable outcomes were documented by van Santen et al., ^[10].

As regard reticulocyte count, there was decrease in its level in group II in comparison to group I and group III, Reticulocyte count, a type of blood test, assesses how quickly the bone marrow produces and releases reticulocytes into the blood. Before they transform to mature red blood cells, reticulocytes remain in the circulation for around 2 days and in IDA there is a low level of iron that is used in manufacturing RBCs and after treatment its level was increased. similar results were reported by Mast et al., ^[11].

In the current research, individuals with IAD had higher levels of IMA, which may be related to hypoxia brought on by low Hb levels. Oxidative stress may be a side effect of iron supplementation. The capacity of the N-terminus of albumin to bond with transition metals may be diminished by reactive oxygen species (ROS), which can be caused by ailments such hypoxia, ischemia, free-radicals, free iron, and acidosis ^[12]

Additionally, mild hypoxia may be the reason for the rise in IMA in anemic individuals, and this hypoxia may also be the cause of the change in MAP during anaemia ^[13].

Significantly negative correlations were found between albumin and IMA. Low albumin content serum samples seem to have higher IMA levels. When there was hypoalbuminemia, the negative correlation was shown to be significant. Several publications have suggested adjustment formulae or ratios to normalize a sample for the albumin content in order to overcome this limiting factor.

Divide IMA by the amount of albumin in g/dL to get the IMA to serum albumin ratio (IMAR) ^[14]. After normalizing our findings, we discovered that group II's IMAR levels were

substantially greater than group I's. IMAR levels peaked after anaemia therapy, much as IMA.

In accordance with Cichota et al.,^[15] findings that substantial correlations between Hb and IMA were discovered in anemic patients with chronic renal disease, our findings showed a strong negative correlation between IMA and Hb.

According to Awadallah et al.,^[16] findings, chronic anaemia, hypoxia, and iron-driven oxidative stresses are the most probable triggers for altering the N-terminus of serum albumin, which results in elevated amounts of IMA in anemic patients.

We have demonstrated that IMA rises during anaemia, and this increase may be related to hypoxia. And their levels were associated with anemia severity. So, IMA may be one of the important markers for detection of the severity of IDA.

Conclusions:

Anemia causes a rise in IMA, and this increase may be related to hypoxia. And we found that the IMA levels could be demonstrative of the severity of anemia.

Its level was elevated after iron therapy (100 mg ferrous glisin sulphate twice daily for two months) attributed to oxidative stress associated with iron therapy, which might alter the structure of albumin and raise IMA values.

Ethical Approval and Consent:

After obtaining permission from Tanta University Hospitals' Ethical Committee, the research was carried out. The patients provided signed permission after being fully informed.

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