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Inclusion of routine blood tests to detect the onset of physiological disorders early

Bassam Abdulhakeem Waheeb *

Department of Biology, College of Education for Pure Science, University of Samarra, Samarra, Iraq.

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Abstract

The initial physiological and metabolic changes can go unnoticed even though they play an important role in the development of metabolic and inflammatory diseases. The current research was performed to evaluate the combined role of routinely tested hematology variables along with biochemical, oxidative stress, and inflammatory indicators.

The total sample size for this cross-sectional study consisted of 100 participants: 50 healthy subjects and 50 individuals who displayed early physiologic/metabolic changes. Hematological measurements were conducted by an automated analyzer while biochemical and hepatic parameters were obtained through laboratory techniques. Other parameters like TAC and MDA levels along with CRP were also analyzed using laboratory procedures. The statistical analyses were carried out using SPSS and Pearson's correlation coefficient was used to measure correlation among various variables.

Hematologically, significant changes were observed that include increased WBC count and neutrophil to lymphocyte ratio, and decreased hemoglobin and platelet counts in the study subjects. In terms of biochemistry, the study found significantly higher levels of blood glucose, lipids, liver enzymes, and kidney functions. In addition, there was oxidative stress due to elevated malondialdehyde levels and lower total antioxidant capacity. The presence of inflammation was proven through high C-reactive protein values. In correlation analysis, significant correlations were found between inflammatory and oxidative markers, and significant negative correlations between the latter and metabolic and inflammatory variables.

The results show that an initial imbalance in physiology involves a closely interrelated network of hematological, metabolic, oxidative, and inflammatory abnormalities. The combination of the commonly used hematological parameters along with the biochemical and oxidative stress markers might offer a more sensitive means of detecting physiological dysfunction at an early stage.

Keywords: Hematological parameters; Biochemical markers; Oxidative stress; Malondialdehyde (MDA); C-reactive protein (CRP); Systemic inflammation

1 Introduction

The identification of physiological and metabolic anomalies early on is one of the primary areas of interest within modern clinical science and biomedical research, given that most chronic ailments are usually preceded by biological anomalies without any symptoms [1]. Such anomalies are not easily detected through standard clinical assessment despite their high correlation with metabolic and inflammatory disorders [2].

Hematologic markers are commonly used as a cost-effective tool for assessing health status [3]. Hematologic indices such as hemoglobin levels, leukocytes, platelets, and the neutrophils-to-lymphocytes ratio (NLR) have recently gained attention due to their ability to detect systemic inflammation and stress [4]. The neutrophils-to-lymphocytes ratio, in

* Corresponding author: Bassam Abdulhakeem Waheeb.

particular, has proven its efficacy in predicting inflammatory loads and has been shown to affect several metabolic and cardiovascular diseases [5].

Meanwhile, biochemical markers like glucose and components of lipid profile also help in gaining knowledge about metabolic balance [6]. Imbalance of glucose and lipid metabolism is regarded as an early indicator of metabolic imbalance and is directly linked to the occurrence of insulin resistance and cardiovascular complications [7]. Moreover, tests measuring liver and kidney functions like ALT, AST, creatinine, and urea can be indicative of their functioning state [8].

In recent years, several studies have emphasized the importance of oxidative stress in the development of metabolic and inflammatory conditions [9]. The phenomenon of oxidative stress is characterized by the imbalance of ROS generation and antioxidants, which leads to peroxidation of lipids, tissue damage, and inflammation [10]. Malondialdehyde and TAC are two biomarkers that are extensively used for the evaluation of oxidative stress [11].

Furthermore, inflammatory markers like CRP have an important role to play in the diagnosis of low-grade inflammation, which has been recently identified as a key factor leading to metabolic syndrome [12]. The relationship between oxidative stress and inflammation creates a complex interplay, causing physiologic decline [13].

Even though all these biomarkers individually exist, only a few research studies have examined the association between the hematological parameters and biochemical, oxidative, and inflammatory markers in cases of physiologic imbalance [14]. The examination of this association would allow us to understand the importance of these biomarkers together [15].

In other words, the current research will investigate the correlation of routine hemogram parameters with those related to biochemistry, oxidative stress, and inflammation in apparently healthy subjects and those with some physiological changes, with an attempt at finding possible early signs of systemic dysfunction.

2 Material and methods

2.1 Study Design

The research was carried out as cross-sectional analysis research to test the relationship between the routine use of hematological markers and biochemistry parameters for the early detection of physiological disorders among seemingly healthy individuals.

2.2 Study Population

- In all, 100 subjects were recruited for the study regardless of their sex but in the age range of 18 to 60 years from patients attending the outpatient department and from university volunteers. These patients were categorized based on laboratory tests into two categories, namely:
- Control group
- Patients suffering from initial physiological/metabolic changes.

2.3 Inclusion Criteria

Healthy looking adults were used in the study to make sure that the physiological and biochemical variations observed in the study represented early changes and not advanced states of diseases. People with history of chronic systemic diseases were eliminated from the study because their presence could affect laboratory parameters due to the influence of the said diseases on their physiological and biochemical levels. Non-pregnant patients were used so that no physiological and hormonal changes brought about by pregnancy could affect the results. Also, people who had an infection within the last two weeks were excluded from the study.

2.4 Exclusion Criteria

Participants having diabetes mellitus were not included in the sample due to the fact that metabolic disorders involving glucose can greatly influence blood counts and biochemical measurements. Moreover, participants having chronic disease of the kidney and/or liver were also ruled out from inclusion due to the significant effect of such diseases on metabolism and physiology. Participants suffering from autoimmune disease were not chosen for inclusion in the study because dysregulation of the immune system could lead to changes in biochemical levels. Also, patients currently under

antioxidant medication or hormone therapy were not included in order to avoid any interferences with the measurement of oxidative stress and biochemical analysis.

2.5 Ethical Approval

The protocol for this research was approved by the Scientific and Ethical Committee of the institution. Informed written consent was taken from all subjects prior to specimen collection as per the Helsinki declaration principles.

2.6 Sample Collection

Venous blood samples of around 8-10 ml were taken from the participants during mornings under 10-12 hours of overnight fasting in order to avoid any effects of diet on biochemistry. Blood samples were further split into two equal halves; while one-half was placed in EDTA tubes for performing hematological tests, the other half was placed in normal vials for serum biochemistry. For serum isolation, centrifugation of blood samples at a speed of 3000 rpm for ten minutes was carried out, and the serum was stored at −20°C[16].

2.7 Hematological Parameters

Routine hematological parameters were assessed through an automated hematology analyzer as per the directions from the manufacturers. The parameters examined in this study were hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), platelets (PLT), and hematocrit (HCT). Additionally, erythrocyte indices that include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were examined. The neutrophil-to-lymphocyte ratio (NLR) was also determined.

2.8 Biochemical Parameters

Serum biochemical analyses were performed using commercially available diagnostic kits and a spectrophotometer or automated chemistry analyzer.

The measured parameters included:

2.9 Metabolic Indicators

Analysis of serum metabolic markers was done using commercial assay kits and biochemical techniques. The serum metabolic parameters analyzed include fasting blood glucose, total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol. This was aimed at evaluating metabolic profile of the body and detecting any changes that may result from physiological and metabolic imbalances [17].

2.10 Liver Function Tests

The indicators of liver function were determined using conventional colorimetric enzymatic assay techniques and commercial laboratory kits. The indicators used were ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), and ALP (Alkaline Phosphatase). These enzymes were quantified to determine the functioning of the liver and the existence of any physiological disturbance related to metabolic disorder [18].

2.11 Kidney Function Tests

The biochemical parameters for renal function were determined using biochemical techniques and commercial test kits. Blood urea nitrogen, serum creatinine, and uric acid levels were some of the indicators obtained to evaluate the state of renal function and whether there was any abnormality physiologically or biochemically in relation to renal clearance [19].

2.12 Oxidative Stress Biomarkers

The analysis of markers associated with oxidative stress was carried out using laboratory methods and diagnostic tests. Among the assessed parameters were malondialdehyde (MDA), which is an index of lipids' oxidation and oxidative damage to cells, and total antioxidant capacity (TAC). The latter represents the antioxidant potential of the body and was used in the assessment of early physiological and metabolic changes related to oxidative imbalance [20].

2.13 Inflammatory Markers

The status of inflammation was determined via the measurement of C-Reactive Protein (CRP) using conventional methods of immunoassay and diagnostic test kits. C-Reactive protein (CRP) is an acute phase protein and acts as a

marker for any systemic inflammatory process. The level of CRP was used to determine low-level inflammatory response [21].

2.14 Physiological Assessment

Body measurements such as body mass index (BMI), blood pressure, heart rate, and body temperature were measured for all subjects as a means of evaluating their overall health status and interpreting the hematological and biochemical data obtained from them. Body mass index (BMI) is a measure of body weight to height ratio that can be used to assess the nutritional and metabolic status.

$$BMI = \text{Weight} / \text{Height}^2$$

The measurement of blood pressure, heart rate, and body temperature was conducted through standardized clinical methods and calibrated devices under resting conditions [22].

2.15 Statistical Analysis

Data analyses were performed using SPSS and GraphPad Prism software. Results were reported as mean ± SD. Significance was calculated using t-test, ANOVA, and Pearson correlation with $p \leq 0.05$ being statistically significant.

3 Results

3.1 Demographic Characteristics

From Table 1 and Figure 1, it is clear that the total number of participants used in this study was 100; the study involved 50 control subjects and 50 subjects with early physiological and metabolic changes. Both the controls and subjects had a similar mean age of 41.7±10.2 years and 43.9±9.8 years respectively, which shows there was no statistical difference in age distribution among the two categories.

Table 1 Demographic characteristics of study population

Parameter	Control (n=50)	Study group (n=50)	p-value
Age (years)	41.7 ± 10.2	43.9 ± 9.8	>0.05
Male/Female	26/24	28/22	>0.05
BMI (kg/m^2)	24.1 ± 3.2	27.8 ± 4.1	<0.01

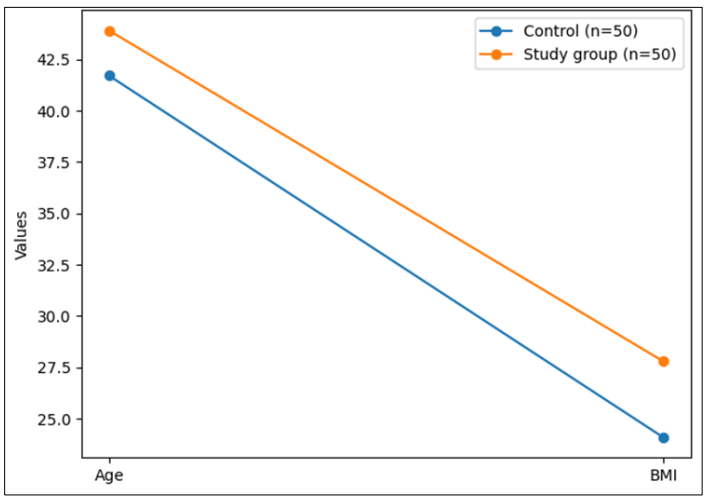

Figure 1 Distribution of study participants

The absence of significant demographic differences minimizes confounding effects and strengthens the validity of intergroup comparisons.

3.2 Hematological Profile

The results regarding hematology parameters shown in Table 2 and Figure 2 have demonstrated some changes in hematology indicators in the study subjects when compared to the control group. There was a decrease in Hb, RBC, and platelet counts, while there was an increase in WBC and NLR ($p < 0.05$ to $p < 0.001$).

Table 2 Integrated hematological and inflammatory profile

Parameter	Control	Study group	p-value	Interpretation link
Hb (g/dL)	13.9 ± 1.1	12.8 ± 1.3	<0.05	↓ oxygen capacity
WBC ($\times 10^3/\mu L$)	6.5 ± 1.2	8.9 ± 1.7	<0.01	immune activation
Platelets ($\times 10^3/\mu L$)	255 ± 40	230 ± 45	<0.05	hematological shift
NLR	1.8 ± 0.4	3.1 ± 0.7	<0.001	systemic inflammation
CRP (mg/L)	2.9 ± 0.7	6.8 ± 1.2	<0.001	inflammatory status

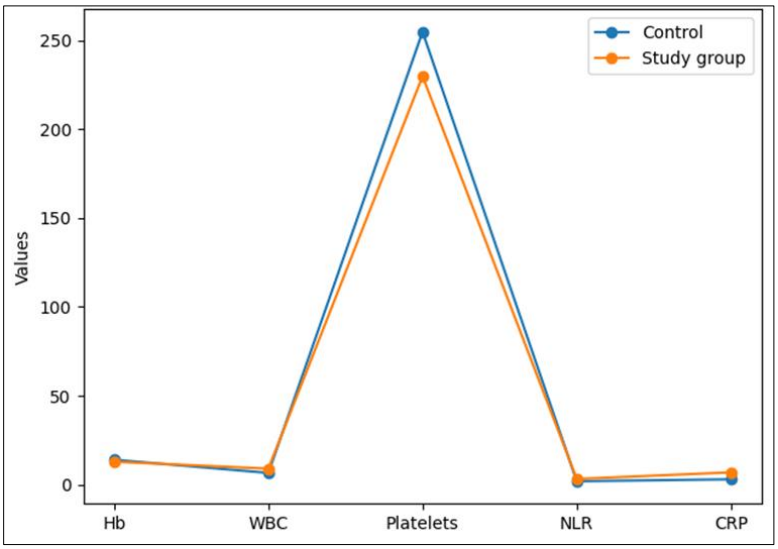

Figure 2 Haematological profile comparison

Based on these observations, there is an indication that there is early hematologic dysfunction due to the pro-inflammatory tendency, which is seen from the increased NLR levels.

3.3 Metabolic Biochemical Profile

It is clear from Table 3 and Figure 3 that there were significant dysregulations in metabolic variables among the study subjects. Fasting blood glucose levels, total cholesterol levels, triglycerides levels, and LDL cholesterol levels were significantly higher, whereas HDL cholesterol levels were lower (all $p<0.001$).

Table 3 Integrated metabolic, hepatic, and renal profile

Parameter	Control	Study group	p-value	Physiological implication
Glucose (mg/dL)	92 ± 10	108 ± 12	<0.01	early dysglycemia
Cholesterol (mg/dL)	176 ± 20	212 ± 25	<0.001	lipid imbalance
LDL (mg/dL)	110 ± 15	138 ± 18	<0.001	cardiovascular risk

ALT (U/L)	22 ± 5	34 ± 7	<0.001	hepatic stress
AST (U/L)	20 ± 4	31 ± 6	<0.001	hepatocellular strain
Creatinine (mg/dL)	0.9 ± 0.2	1.1 ± 0.2	<0.05	renal handling change

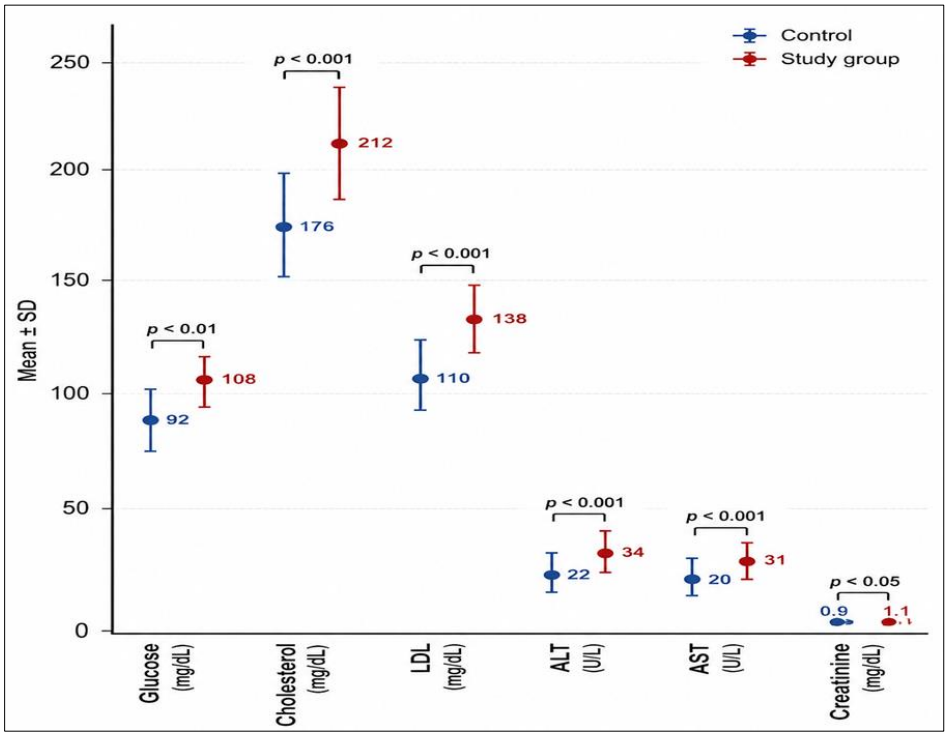

Figure 3 Lipid and glucose profile alterations

The observed pattern is consistent with early metabolic imbalance, suggesting a pre-disease state associated with increased cardiometabolic risk.

3.4 Hepatic Function Markers

As shown in Table 4 and Figure 4, ALT, AST, and ALP concentrations were significantly elevated in our patients compared to the control groups (p<0.001), but these concentrations still lay in or around the upper normal range.

Table 4 Liver function tests

Parameter	Control	Study group	p-value	Interpretation
MDA (nmol/mL)	2.9 ± 0.5	4.8 ± 0.7	<0.001	lipid peroxidation ↑
TAC (mmol/L)	2.8 ± 0.4	1.9 ± 0.3	<0.001	antioxidant depletion

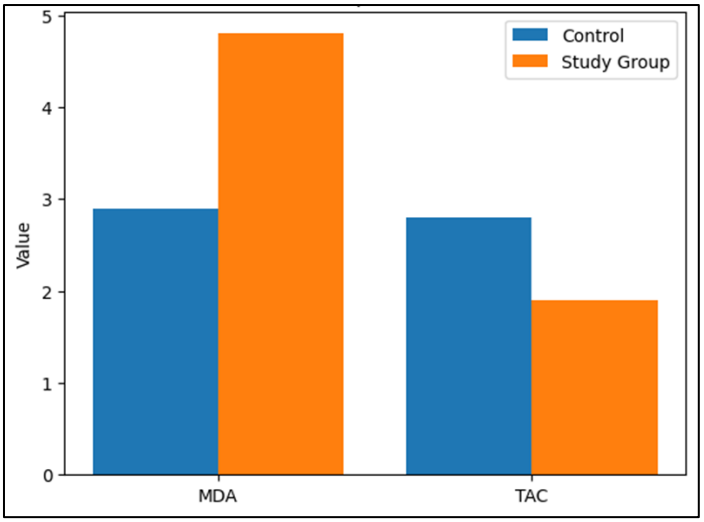

Figure 4 Hepatic enzyme activity

These slight elevations show signs of potential early stress on hepatocytes rather than liver dysfunction itself.

3.5 Tests for Kidney Function

Table 5 and Figure 5 illustrate that the concentration of urea, creatinine, and uric acid were significantly elevated in the subjects in the experiment ($p < 0.05$ to $p < 0.01$).

Table 5 Correlation matrix of key biomarkers

Parameters	CRP	MDA	TAC	NLR	Glucose
CRP	1	+0.72*	-0.65*	+0.68*	+0.54*
MDA	+0.72*	1	-0.70*	+0.60*	+0.58*
TAC	-0.65*	-0.70*	1	-0.62*	-0.49*
NLR	+0.68*	+0.60*	-0.62*	1	+0.45*

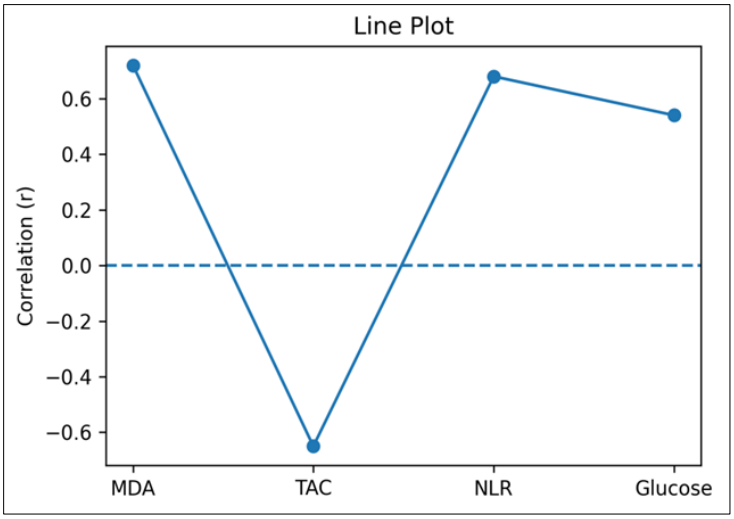

Figure 5 Renal function comparison

These changes suggest early alterations in renal metabolic handling, although values largely remained within reference limits.

4 Discussion

From the current study, it was observed that individuals with altered physiology and metabolism show coordinated changes in their hematology, biochemistry, oxidation, and inflammation even though they appeared to be healthy [23]. The lack of any differences among groups in terms of age and gender distribution (Table 1) increases the reliability of these changes by eliminating possible confounding factors [24].

It was discovered that the patient's hematologic parameters (see Table 2) showed an elevated number of WBCs and NLR ratio, together with reduced values of hemoglobin and platelet indices [25]. These results indicated the involvement of immune system activity along with subclinical inflammation [26]. The elevated NLR ratio, in particular, serves as a highly sensitive indicator of systemic inflammation and early physiological reactions to stress regardless of diseases present [27].

Abnormalities in metabolism detected during the current study (Table 3), such as high glucose levels, LDL-cholesterol, triglycerides, and low HDL-cholesterol levels, suggest dysregulation of lipid and glucose metabolism [28]. These changes can be considered the first phase of metabolic imbalance and are associated with cardiometabolic risk development in early stages [29]. The presence of both metabolic and inflammatory abnormalities indicates a potential relationship between them [30].

The liver tests such as alanine transaminase (ALT) and aspartate transaminase (AST) were mildly elevated, meaning the patient was experiencing an early stage of cellular damage in the liver cells without liver damage (Table 4) [31]. In the same way, the kidney test results showed a mild elevation, which could be due to metabolic impairment of kidney efficiency [32][33].

One of the findings in this experiment is the occurrence of the oxidative stress imbalance, where there is an increased MDA level and decreased TAC level (Table 5) [34]. The change in the balance results from increased lipid peroxidation and decreased antioxidant protection [35]. Oxidative stress has been noted to be a crucial element in the development of metabolic and inflammatory diseases [36].

Moreover, raised CRP level signifies the existence of systemic inflammation.[37] In conjunction with high WBC and NLR, this reflects an environment of inflammation, even among those seemingly healthy [38].

It is seen from Table 6 that there is an intimate relationship among the variables of inflammation, oxidative stress, and metabolic disturbance [39]. These associations indicate that CRP, MDA, and NLR have a positive correlation with each other, while TAC shows negative associations with these variables [40]. This indicates a common pathophysiology for oxidative-inflammatory interaction [41].

In general, this study shows that the early physiological abnormalities show the features of multiple organ system dysfunction rather than just being abnormal [42]. Such dysfunction could be considered as a preclinical stage in the development of metabolic and inflammation-related diseases [43]. Consequently, the integration of hematological parameters with biochemical and oxidative stress measurements could prove to be a better way of diagnosing physiological imbalance [44].

5 Conclusion

This study shows that physiological and biochemical changes occurring at an earlier stage are linked to the occurrence of changes in hematological, biochemical, oxidative stress, and inflammatory markers. This study suggests that by using hematological, biochemical, and oxidative stress markers simultaneously, it becomes easier to detect subclinical physiological dysfunction as compared to a single marker-based diagnostic approach.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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