



Relationship Between Oxidative Stress and Chromosomal Damage in Patients with Chronic Diseases

A case-control study at Al-Hussein Teaching Hospital, Al-Samawah City, Al-Muthanna Governorate, Iraq

Shaimaa J AL Sabagh

Department of Dialysis, polytechnic college-ALSamawah, AlFurat AIAwsat technical university

Abstract

Chronic non-communicable diseases are identified by the prolonged imbalance in the production of reactive oxygen species and antioxidant defense mechanism. Reactive species can directly attack DNA, and if there are no repairs of the lesions, these can lead to the identification of chromosomal damage, which is a well-established indicator of genomic stability and cancer risk. There is less data related to this relationship in the context of Iraq.

The aim of the study was to examine the oxidative stress evaluation and chromosomal damage among patients with type 2 diabetes mellitus (T2DM), chronic kidney disease (CKD) and essential hypertension admitted at Al-Hussein Teaching Hospital from Al-Samawah city of Iraq.

A case-control study was undertaken on 120 patients (40 T2DM, 40 non-dialysis CKD stages 3-4 and 40 essential hypertension patients), as well as on 40 control patients matched by age and sex. The study determined the level of serum malondialdehyde (MDA), total oxidant status (TOS), total antioxidant capacity (TAC), reduced glutathione (GSH), and activity of superoxide dismutase (SOD). The designed oxidative stress index (OSI) was also calculated. Chromosomal damage was evaluated among cultured peripheral blood lymphocytes using the metaphase chromosomal aberration (CA) technique and the cytokinesis-block micronucleus (CBMN) method.

Findings: All patient groups showed higher amounts of MDA, TOS and OSI and lower amounts of TAC, GSH and SOD than controls ($p < 0.001$), most notably in CKD. The frequency of total CA (CKD $4.5 \pm 1.4\%$, T2DM $3.6 \pm 1.2\%$, hypertension $2.8 \pm 1.0\%$ vs $1.2 \pm 0.6\%$ in controls) and micronuclei (18.2, 14.9 and 12.1 vs 6.8 per 1000 binucleated cells respectively) was significantly increased ($p < 0.001$). In the overall study group, OSI showed a direct correlation with the frequency of micronuclei ($r = 0.66$) as well as the frequency of CA ($r = 0.59$) and an inverse correlation with TAC ($r = -0.49$ and -0.43). After adjustment for age, sex, smoking, BMI and the duration of the disease OSI remained the strongest independent factor for the frequency of micronuclei in patients ($\beta = 0.41$, $p < 0.001$).

Oxidative stress is highly correlated with chromosomal defects in patients suffering from chronic diseases. Routine evaluation of oxidative stress as well as cytogenetic indicators may facilitate the identification of patients in whom the probability of genome instability is the highest.

Keywords:

oxidative stress; chromosomal aberrations; micronucleus; malondialdehyde; total antioxidant capacity; type 2 diabetes mellitus; chronic kidney disease; hypertension; Iraq.

Introduction

Chronic non-infectious diseases (NCDs)—including diabetes, chronic kidney disease, hypertension, and other cardiovascular disorders—are now the most prevalent diseases causing morbidity and mortality across the globe. The World Health Organization has stated that NCDs are responsible for most of the deaths taking place all over the world, and countries with lower gross domestic product (GDP) are increasingly reporting cases of these diseases; this includes countries in the Eastern Mediterranean Region where Iraq is located [1]. Diabetes alone affects millions of people in the world and its rate in the Middle East and North Africa is among the highest; unfortunately, the majority of cases are diagnosed only when the complications arise [2]. In the case of Iraq, the epidemiological transition has been hastened by the process of urbanization as well as the changes in diet, lack of physical activity, smoking, and wars that have affected the health care system and exposed the population to pollution. Chronic diseases are prevalent in southern provinces, especially in Al-Muthanna province, the capital of which is Al-Samawah; however, very little is known about the cellular and molecular processes occurring because of chronic diseases.

Oxidative stress is a common biochemical trait, appearing to be present in many chronic illnesses. According to Sies, oxidative stress is the condition where oxidants are dominant over antioxidants. It leads to problems in redox signalling and/or molecular damage [3]. Later developments of this concept separated the terms oxidative “oxidative eustress” (the presence of small amount of oxidants), and oxidative “oxidative distress” (large amounts of oxidants causing damage to biomolecules) [4]. Reactive oxygen species (ROSs) such as superoxide anion, hydrogen peroxide and hydroxyl radical are produced in cells, along with reactive nitric species like peroxynitrite. The key endogenous sources are mitochondrial chain of electron transport, NADH oxidases, xanthine oxidase, uncoupled nitric oxide synthase, cytochrome P450 enzymes and active phagocytes [5]. Under normal circumstances these species are perfectly controlled [5,6].

The antioxidant system of the body protects cells from the excess of ROS. There are enzymatic systems, such as superoxide dismutase (SOD), which converts superoxide to hydrogen peroxide, followed by catalase or glutathione peroxidase that reduce hydrogen peroxide to water, while glutathione reductase will regenerate reduced glutathione (GSH). The body has a non-enzymatic antioxidant system, which includes GSH as the primary intracellular source for thiols, and also vitamins C and E, uric acid, albumin thiols, bilirubin and dietary polyphenols [5,7]. If ROS production exceeds the capacity of antioxidants or if their capacity is consumed, oxidative modification of lipids, proteins, carbohydrates, and nucleic acids will occur. Lipid peroxidation of polyunsaturated fatty acids in membranes produces reactive aldehydes, with malondialdehyde (MDA) being the one most often used as a clinical marker. Because of interaction of individual antioxidants and compensation of their roles, an integrated measure of total antioxidant capacity (TAC) and total oxidant status (TOS) was developed, as well as the oxidative stress index (OSI) that can quantify the relationship between these parameters and enable an overview of redox state in clinical samples [8].

Among the various cellular targets, DNA occupies an unique position in biological systems as the damage done to DNA can be passed on to the progeny cells. Hydroxyl radical reacts with various constituents of DNA compromising the DNA integrity giving rise to oxidized bases, abasic sites, and single and double-strand breaks [9]. One of the most commonly investigated oxidative lesions, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG or 8-OHdG), can lead to mispairing with adenine during the replication process resulting in G:C to T:A transversions. In recent times, it has been used as an useful marker of oxidative damage in DNA, and it has also been associated with the process of cancer development and degenerative diseases [9,10]. Lipid peroxidation products including MDA and 4-hydroxynonenal have also been reported to produce exocyclic DNA adducts which are mutagenic in nature thus establishing an indirect mechanism through which oxidation of membranes poses a threat to the integrity of genome. In response to damage to DNA, cells give rise to a regulated DNA damage response mechanism which detects the damage and initiates the arrest of the cell cycle in addition to activating repair mechanisms like base excision repair, nucleotide excision repair, homologous recombination, and non-homologous end joining [11]. In instances when there is extensive damage or impaired capacity for repair, the damaged sites fail to undergo repair or misrepair and accumulate leading to mutation, cellular senescence, apoptosis, aging, and cancer [12].

At the chromosome level, the persistent damage in the DNA takes the form of structural chromosomal aberrations (CA) and micronuclei (MN). Chromosomal aberrations, which include chromatid and chromosome breaks, acentric fragments, dicentric and ring chromosomes, and exchanges can be observed directly under the microscope at the metaphase stage of cultured lymphocytes. Micronuclei are tiny bodies that exist outside of the nucleus and contain acentric chromosome fragments or whole chromosomes that do not get incorporated into the main nucleus during anaphase. The cytokinesis-block micronucleus (CBMN) cytome assay, which uses cytochalasin-B to identify cells that have undergone one cell division, allows not only for MN to be evaluated, but also for nucleoplasmic bridges (NPB) and nuclear buds (NBUD) at the same time with other measures of cytotoxicity and proliferation [13]. By standardizing this technique through the HUMN project (the acronym for the international Human MicroNucleus), it becomes one of the most reliable tools for measuring genome damage that has occurred during human biomonitoring [14]. Nucleoplasmic bridges indicate the presence of dicentric chromosomes which have resulted from erroneous repair of breaks in the DNA or the connections that were made at the ends of the two strands of DNA, while nuclear buds are indicative of eliminating any amplified DNA or unfixable DNA repair units. In this way, the cytome technique allows for identifying and quantifying different causes of chromosomal instability [15].

The clinical significance of these cytogenetic endpoints is corroborated by the prospective cohort study findings. The large Nordic and Italian cohort studies showed that individuals exhibiting higher frequency of chromosomal aberrations in peripheral blood lymphocytes had significantly elevated risk of developing cancer even in the absence of exposure to recognized carcinogens [16]. Likewise, results of a pooled analysis from HUMN database report elevation in frequency of micronucleus in lymphocytes as predictor for the risk of cancer in healthy individuals [17]. The studies indicate that lymphocyte chromosomal damage is a marker of genomic instability rather than merely an evidence of exposure to risk factors. Oxidative stress also impacts telomeres since telomeres are particularly susceptible to oxidative damage because of the guanine-rich sequence along with inefficiency of repair. Under conditions of oxidative stress, telomere shortening will also accelerate by promoting the bridge-fusion-breakage cycles [18]. Studies conducted using human cells have shown that continuous exposure to low levels

of oxidative agents leads to chromosomal instability which persists through several generations even after initial insult [19].

Type 2 diabetes mellitus (T2DM) serves as an ideal example of persistent oxidative stress. The effect of hyperglycaemia is manifested by higher mitochondrial production of the superoxide ion and subsequent activation of the polyol and hexosamine pathways, protein kinase C isoforms and advanced glycation end product (AGE) formation, thereby linking elevated glucose levels to diabetic complications in both micro- and macrovasculature [20,21]. Glycooxidation and AGE–receptor interaction increase ROS production further. Several studies indicate a rise in micronucleus production in lymphocytes of diabetic patients, and a review of the literature concludes about the increase of MN in obesity, diabetes and cardiovascular disease which have in common oxidative and inflammatory pathways [22]. Evidence that folate can affect micronucleus production indicates that part of the impact is modifiable [23].

Chronic kidney disease (CKD) is associated with an even more pronounced pro-oxidant state. Retention of uraemic toxins, reduced clearance of oxidised products, chronic inflammation, anaemia, iron therapy and, in later stages, the bioincompatibility of dialysis all contribute to oxidative stress, which has been described as a unifying concept linking uraemia with its high cardiovascular risk [24]. Antioxidant defences, particularly the glutathione system and selenium-dependent glutathione peroxidase, are depleted as renal function declines [25]. Patients with end-stage renal failure exhibit increased genomic damage, including elevated micronucleus frequencies and DNA strand breaks, which has been attributed in part to AGEs and carbonyl stress [26]. A broader evaluation of clinical biomarkers confirmed that DNA damage rises with progressive loss of kidney function and may contribute to the increased cancer incidence observed in this population [27].

Essential hypertension and the related spectrum of cardiovascular diseases are also very much linked to oxidative stress. The nitrogen oxides include vascular NADPH oxidases, uncoupled nitric oxide synthase in endothelial cells as well as mitochondria that cause generation of free radicals that will reduce the bioavailability of nitric oxides, improve the occurrence of endothelial dysfunction, vascular smooth muscle cell proliferation, and vascular remodeling [28]. Oxidative stress is now being recognized as the common link in various mechanisms of hypertension, including the activation of the renin-angiotensin-aldosterone system [29]. In coronary artery disease patients, there is a positive correlation between increased micronuclei and adverse events in the future, meaning that chromosome injury may also have prognostic value apart from cancer [30].

Oxidative stress usually does not play a role itself. Indeed, the phenomenon can be seen in connection with chronic low-grade inflammation, when activated immune cells release ROS and produce other substances that contribute to the generation of oxidants, creating a self-feeding process involved in the formation of tumors [31]. Metabolic inflammation, or “metaflammation”, connects the issues of obesity and insulin resistance with the other diseases discussed in this paper [32]. Ageing complicates this process as oxidative damage accumulates, while the capacity of the organism to produce antioxidants and repair itself goes down with age, making older patients more vulnerable to chronic diseases and genomic instability [33].

The specific links between many chronic diseases and either oxidative stress or chromosomal injury are well-illustrated, but relatively few studies have tended to analyze both sets of biomarkers on the same people and check whether the degree of redox imbalance actually allows calculating the scale of the cytogenetic damage in different types of diseases. Local data from Iraq where the population exposure, nutrition and healthcare vary considerably from those

of the populations covered by the majority of research is very limited. Exploring this relationship could pave the way for the use of oxidative and cytogenetic biomarkers for risk assessment and for the development of antioxidant approaches and lifestyle interventions.

The current research aims to evaluate the oxidative stress biomarkers which are (MDA, TOS, TAC, OSI, GSH, and SOD) along with chromosomal damage in patients suffering from T2DM, CKD, and essential hypertension and attending Al-Hussein Teaching Hospital in Al-Samawah city, Iraq, and compare the results with healthy controls, in addition to analyzing whether oxidative stress has a prospective independent association with chromosomal damage after adjusting to applicable sociodemographic and clinical variables.

Materials and Methods

Study design and setting

This study was a hospital-based, observational case–control experiment conducted in the Consultation Clinics and Internal Medicine Department of Al-Hussein Teaching Hospital located in Al-Samawah city of Al-Muthanna Governorate in southern Iraq. As a matter of fact, this hospital is the largest public referral hospital of the governorate serving both the urban population of Al-Samawah and other nearby rural areas. The laboratory analysis took place in the biochemistry lab of the hospital and cytogenetics lab of the university collaborating with the study. Data collecting occurred throughout twelve consecutive months from November 2023, October 2024, which ensured that seasonal variations in dietary antioxidant intake would have been equally distributed among the groups.

Study population and sample size

The study included 160 subjects distributed in groups of 40 people in each group. In group I, patients had type 2 diabetes mellitus (T2DM) diagnosed according to the criteria of the American Diabetes Association for at least five years. The participants in group II were patients with chronic kidney disease (CKD) stage 3–4, defined as patients with an estimated glomerular filtration rate (eGFR) of 15–59 mL/min/1.73 m² who had had their condition for more than three months according to the KDIGO criteria and who had not started renal replacement therapy. Group III had patients with essential hypertension diagnosed for at least five years who were on antihypertensive treatment but had no diabetes or renal failure. Group IV consisted of healthy individuals recruited from the hospital staff, blood donors, and accompanying relatives matched to the patient groups in terms of age (± 5 years), gender, and smoking status.

The sample size is estimated by using the equation for comparison between the two means, assuming an anticipated discrepancy of 0.5 standard deviations in micronucleus frequency between controls and patients according to literature data, $\alpha=0.05$ and statistical power of 80%, which resulted in the minimum required n of 34 per group, being increased to 40 to account for culture failure and incomplete information. Selection of participants took place via consecutive non-probabilistic sampling of eligible individuals until reaching a sufficient quota.

Eligibility criteria

Both male and female participants aged 35–65 years were eligible for the study. Exclusions were made for patients with any active or past cancer, chemotherapy, or radiation treatment at any time, or transformed for ionizing radiation diagnostic imaging during the last three months, an acute infection, or febrile condition in the last four weeks, chronic viral condition (hepatitis

B, hepatitis C, or HIV), autoimmune disease or inflammatory rheumatic condition, as well as being pregnant or nursing. Participants were also excluded if they typically consumed antioxidant vitamins (vitamin C, vitamin E, selenium or N-acetylcysteine) during the previous three months, or had a known job exposure to pesticides, heavy metals, benzene or solvents. Controls were expected to have fasting plasma glucose under 100 mg/dL, blood pressure under 140/90 mmHg, normal kidney function, and no use of chronic medication.

Ethical considerations

The Research Ethics Committee at Al-Muthanna Health Directorate reviewed and approved the research protocol thoroughly and in conjunction with the scientific committee at the collaborating college, in addition to obtaining formal administrative approval from the authorities at Al-Hussein Teaching Hospital. The study was conducted in line with the principles of the Declaration of Helsinki. Informed written consent was obtained from all participants after explaining the purpose of the study, the procedures involved, the voluntary nature of participation and confidentiality of the study in Arabic. Participation was voluntary and participants were free to withdraw from the study at any stage without affecting their medical care.

Clinical and anthropometric assessment

A structured questionnaire was filled up for every participant through face to face interview, including questions about age, gender, locality, educational level, job, smoking behavior, family background of critical disease, duration of the illness and medicines taken. Weight and height measurements of the participants were taken while they were in their light clothes and barefoot, and the BMI was determined by dividing other measurement result in kilograms by the height in meters to the power of two. Blood pressure was measured twice while participants were seated after receiving at least ten minutes' rest with the help of a calibrated mercury sphygmomanometer, and the arithmetic mean was calculated.

Blood sampling

Ten millilitres of venous blood were collected from the antecubital vein after fasting for ten to twelve hours, using disposable, sterile syringes, and minimal stasis. Five millilitres were put into plain gel tub and left to clot for twenty minutes at room temperature before centrifugation at 3000 rpm for ten minutes. Serum was separated and kept in aliquots in Eppendorf tubes and stored at minus 20 – °C until analysis to avoid repeated freeze-thaw cycles. Two millilitres of blood were collected in EDTA tubes to assess glycated hemoglobin (HbA1c) and hematological parameters while the remaining three millilitres were collected in sterile sodium heparin tubes for lymphocyte culture with heparinized samples being processed within four hours at room temperature.

Routine biochemical analyses

The measurements of fasting plasma glucose, serum urea, creatinine, uric acid, total cholesterol, triglycerides, high-density lipoprotein cholesterol, and total protein were carried out using a commercial enzymatic kit and the manufacturer's controls and calibrators on an automated clinical chemistry analyzer. The Low-density lipoprotein cholesterol level was calculated by using the Friedewald equation. HbA1c was measured by the method of ion-exchange chromatography. The estimated glomerular filtration rate was calculated using the CKD-EPI

creatinine formula. For internal quality control purposes, control material was analyzed at two concentrations in the current runs, and the runs were considered valid only if the control values of the current run were within ± 2 standard deviations of the assigned mean.

Oxidative stress biomarkers

The process of lipid peroxidation was measured through malondialdehyde (MDA) concentration in serum with the thiobarbituric acid reactive substances technique. Serum sample was treated with thiobarbituric acid at acidic conditions for 60 minutes at 95 °C, followed by reading of the absorbance (pink color formation) at 532 nm against the standard curve of 1,1,3,3-tetraethoxypropane. Data was presented in terms of $\mu\text{mol/L}$. The total antioxidant capacity (TAC) and total oxidant status (TOS) were determined using the standardized colorimetric assay kits based on the automated methods described by Erel in which the TOS was expressed in $\mu\text{mol H}_2\text{O}_2$ equivalent/L and the TAC was provided in terms of mmol Trolox equivalent/L. The oxidative stress index (OSI) was represented as the ratio between the TOS and TAC after the conversion of TAC to $\mu\text{mol/L}$. The measurement of glutathione (GSH) in serum was performed using the Ellman's technique (by employing 5,5'-dithiobis-(2-nitrobenzoic acid)), where the absorbance was measured at 412 nm and results obtained in terms of $\mu\text{mol/L}$. The determination of SOD (Superoxide dismutase) activity was executed using the pyrogallol autoxidation technique with a single unit defined as the enzyme quantity which reduces pyrogallol autoxidation by 50%, with results presented as U/mL. The readings of absorbance were obtained using calibrated spectrophotometer. The samples were run in duplicates, the average being pursued, while tests with a variability coefficient greater than 10% were repeated.

Lymphocyte culture and chromosomal aberration analysis

In the conventional cytogenetic study, 0.5 ml of heparinized whole blood was combined with 5 ml of RPMI-1640 solution endowed with 20% fetal bovine serum, L-glutamine, penicillin-streptomycin and 0.1 ml of phytohaemagglutinin, and heated at 37 °C for 72 hours. The last two hours involved adding colchicine (0.05 $\mu\text{g/ml}$) to stop cells at metaphase division. Then, centrifugation was performed to collect cells in the growth medium, which were treated with warm hypotonic 0.075 M potassium chloride for 20 minutes at 37 °C and subsequently fixed in freshly made methanol-glacial acetic acid (3:1) three times. Slides were produced by applying the cells to prepared slides on blue melted ice. Slides were air dried and stained in 5% Giemsa solution in phosphate buffer (pH 6.8) for 10 minutes. Each participant's metaphase was examined at $\times 1000$ magnification under immersion oil. The type of breaks were classified following the International System of Human Cytogenetics by chromatid and chromosome breaks, acentric material, decipher chromosomes and chromatid exchanges while gaps were documented separately and were not included in aberrations figure. The results appeared as percentages of aberrant cells and the numbers of aberrations for every 100 cells counted.

Cytokinesis-block micronucleus assay

Cultures of cells were created under the same set of conditions and cytochalasin-B was added at a concentration of 6 $\mu\text{g/mL}$ exactly 44 hours after culturing. Cultures were harvested 72 hours later and were subsequently treated in mild hypotonic condition using 0.075 M KCl and fixed in methanol-acetic acid where first fix was made at lower temperature to preserve the cytoplasm of the cultures and staining carried out using Giemsa. For counting purposes, 1000 binucleated cells with intact cytoplasm were selected for each of the participants using HUMN

project protocol in terms of counting micronuclei (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBUD). The calculation of nuclear division index (NDI) was based upon 500 viable cells as $[M1 + 2(M2) + 3(M3 + M4)]/N$, where categories are present namely M1–M4 which correspond to cells with nucleus to nuclei that can be present. All slides decoded and evaluated in a blind fashion by two experts; conflicting cells evaluated jointly for resolving the conflict and agreement between the two was checked on a random sample of slides.

Statistical analysis

SPSS version 26 was employed to perform data analysis. Normality testing was performed using the Shapiro-Wilk test. The results for normally distributed variables are expressed as mean $\pm$ standard deviation, while skewed variables are reported as median with interquartile range and categorical outcomes are reported as frequencies and percentages of issues. To determine difference between the four groups studied, one-way analysis of variance with Tukey's post hoc test was applied or a Kruskal-Wallis test in conjunction with Mann-Whitney U test with Bonferroni correction procedure used in the case of skewed variables. Chi-square test of independence was applied to categorical variables under investigation. The correlations between oxidative stress markers and cytogenetic endpoints were evaluated using Pearson or Spearman correlation as appropriate. To define independent predictors of frequency of micronuclei, multiple linear regression analysis was applied with age, gender, smoking, body mass index, duration of the disease and OSI being included in the covariance analysis. Two-sided p value less than 0.05 was considered statistically significant.

Results

In total, there were 160 enrolled participants in the study, all of whom completed it successfully, with cultures obtained in all cases. The four groups were comparable in terms of age, sex and smoking status confirming good matching; however, being in the diabetic group was associated with higher body mass index, while the hypertensive group had the highest systolic blood pressure levels as indicated in Table 1. Average duration of the disease was found to be 9.4 ± 3.6 years in T2DM, 6.1 ± 2.8 years in CKD and 10.2 ± 4.1 years in hypertension.

Table 1. Demographic, anthropometric and routine laboratory characteristics of the study groups (mean $\pm$ SD)

Variable	Control (n = 40)	T2DM (n = 40)	CKD (n = 40)	Hypertension (n = 40)	p value
Age (years)	51.3 $\pm$ 8.1	53.6 $\pm$ 7.9	54.8 $\pm$ 8.4	53.1 $\pm$ 8.0	0.244
Male / Female (n)	22 / 18	21 / 19	23 / 17	22 / 18	0.971
Smokers, n (%)	11 (27.5)	13 (32.5)	12 (30.0)	14 (35.0)	0.891
BMI (kg/m ²)	26.1 $\pm$ 3.0	30.4 $\pm$ 4.1	27.5 $\pm$ 3.6	29.2 $\pm$ 3.9	< 0.001
Systolic BP (mmHg)	120.4 $\pm$ 8.6	131.2 $\pm$ 11.0	142.6 $\pm$ 13.4	149.8 $\pm$ 12.1	< 0.001
Fasting glucose (mg/dL)	89.6 $\pm$ 8.3	186.4 $\pm$ 42.7	104.2 $\pm$ 15.6	97.3 $\pm$ 11.2	< 0.001
HbA1c (%)	5.2 $\pm$ 0.4	8.6 $\pm$ 1.5	5.8 $\pm$ 0.6	5.5 $\pm$ 0.5	< 0.001
Serum creatinine (mg/dL)	0.84 $\pm$ 0.15	1.02 $\pm$ 0.21	2.76 $\pm$ 0.84	0.96 $\pm$ 0.18	< 0.001

eGFR (mL/min/1.73 m ²)	97.8 ± 11.4	86.3 ± 13.9	34.7 ± 10.2	83.5 ± 12.6	< 0.001
Total cholesterol (mg/dL)	178.2 ± 26.4	213.5 ± 34.8	205.9 ± 33.1	201.4 ± 30.7	< 0.001

Oxidative stress indices were significantly different in both samples (Table 2). The serum levels of MDA, TOS and OSI were found to be significantly elevated while TAC, GSH and SOD were for sure found to be significantly decreased in all groups of patients when compared to controls; ($p < 0.001$).

The indices were discovered to be at their highest level in CKD patients, where OSI is found to be three times higher than control group value, followed by T2DM and hypertension patients. Post-hoc testing revealed that CKD samples were significantly different from both T2DM and hypertension samples concerning MDA, TOS, OSI and GSH, whereas the difference between T2DM and hypertension concerning MDA and OSI reached significance but not SOD.

Table 2. Oxidative stress and antioxidant biomarkers in the study groups (mean ± SD)

Biomarker	Control	T2DM	CKD	Hypertension	p value
MDA (μmol/L)	1.84 ± 0.42	3.76 ± 0.88	4.62 ± 1.05	3.11 ± 0.74	< 0.001
TOS (μmol H ₂ O ₂ Eq/L)	11.6 ± 3.2	22.4 ± 5.8	27.9 ± 6.9	19.1 ± 4.7	< 0.001
TAC (mmol Trolox Eq/L)	1.52 ± 0.24	1.08 ± 0.21	0.86 ± 0.19	1.19 ± 0.23	< 0.001
OSI (arbitrary unit)	0.78 ± 0.26	2.14 ± 0.68	3.38 ± 0.97	1.65 ± 0.52	< 0.001
GSH (μmol/L)	32.6 ± 5.4	23.1 ± 4.8	18.4 ± 4.2	26.3 ± 5.1	< 0.001
SOD (U/mL)	14.8 ± 2.6	10.2 ± 2.3	8.7 ± 2.1	11.4 ± 2.4	< 0.001

Cytogenetic studies revealed a parallel gradient of injuries (Table 3). The total rate of chromosomal aberrations excluding gaps was 1.2 ± 0.6 aberrations per 100 cells for controls and increased to 2.8 ± 1.0 for hypertension, 3.6 ± 1.2 for T2DM, and 4.5 ± 1.4 for CKD patients. Chromatid breaks were identified as the solitary of then most frequent aberration in all groups, where as dicentric and ring chromosomes were rare but almost only occurred among patients. The appearance rate of micronuclei per 1000 binucleated cells increased from 6.8 ± 2.1 for controls to 12.1 ± 3.4 , 14.9 ± 4.0 and 18.2 ± 4.6 for hypertension, T2DM and CKD respectively. The same was seen in the frequency of nucleoplasmic bridges and nuclear buds, while the nuclear division index was reduced significantly at CKD as compared to controls indicating slower lymphocyte proliferation.

Table 3. Chromosomal aberrations and micronucleus cytome parameters (mean ± SD)

Parameter	Control	T2DM	CKD	Hypertension	p value
Aberrant cells (%)	1.1 ± 0.5	3.2 ± 1.1	4.0 ± 1.3	2.5 ± 0.9	< 0.001
Total aberrations / 100 cells	1.2 ± 0.6	3.6 ± 1.2	4.5 ± 1.4	2.8 ± 1.0	< 0.001
Chromatid breaks / 100 cells	0.7 ± 0.4	2.0 ± 0.8	2.4 ± 0.9	1.6 ± 0.6	< 0.001
Chromosome breaks / 100 cells	0.3 ± 0.2	0.9 ± 0.5	1.2 ± 0.6	0.7 ± 0.4	< 0.001
Dicentrics + rings / 100 cells	0.02 ± 0.05	0.21 ± 0.14	0.34 ± 0.19	0.14 ± 0.11	< 0.001
Micronuclei / 1000 BN cells	6.8 ± 2.1	14.9 ± 4.0	18.2 ± 4.6	12.1 ± 3.4	< 0.001

Nucleoplasmic bridges / 1000 BN	1.2 ± 0.8	3.1 ± 1.4	4.0 ± 1.7	2.4 ± 1.1	< 0.001
Nuclear buds / 1000 BN	2.4 ± 1.1	5.2 ± 1.9	6.3 ± 2.2	4.1 ± 1.6	< 0.001
Nuclear division index	1.86 ± 0.14	1.74 ± 0.16	1.66 ± 0.18	1.79 ± 0.15	< 0.001

Correlation analysis conducted on the combined patient samples (n = 120) verified a significant correlation between the redox imbalance and the genomic injury (Table 4). OSI demonstrated the most noteworthy positive correlation with the micronucleus (r = 0.66) and numerically more aberrations (r = 0.59) than did MDA and TOS. In contrast, TAC, GSH, and SOD showed non-positive correlations with both parameters. Within the different diseases, correlations were strongest for CKD (OSI with MN, r = 0.71) and weakest for hypertension (r = 0.54). HbA1c was positively correlated with micronucleus in diabetes (r = 0.52, p < 0.001), while eGFR was negatively correlated with MN in CKD (r = -0.57, p < 0.001).

Table 4. Correlation of oxidative stress biomarkers with cytogenetic damage in pooled patients (n = 120)

Biomarker	r with total aberrations	p	r with micronuclei	p
MDA	0.54	< 0.001	0.61	< 0.001
TOS	0.51	< 0.001	0.57	< 0.001
OSI	0.59	< 0.001	0.66	< 0.001
TAC	-0.43	< 0.001	-0.49	< 0.001
GSH	-0.46	< 0.001	-0.52	< 0.001
SOD	-0.38	< 0.001	-0.44	< 0.001

Using micronucleus frequency as the dependent variable, the strongest predictor in multiple linear regression was OSI (standardised β = 0.41, p < 0.001). Age (β = 0.22, p = 0.004), duration of disease (β = 0.19, p = 0.011) and smoking (β = 0.16, p = 0.028) came in second, third and fourth respectively while sex and BMI showed no significant effect. The multiple regression model accounted for 54% of the variability in micronucleus frequency (adjusted R^2 = 0.54, p < 0.001). The inter-observer agreement for scoring micronucleus was found to be very good with a high intraclass correlation coefficient (0.92).

Discussion

The central finding of this study is that patients with chronic diseases attending Al-Hussein Teaching Hospital in Al-Samawah carry a measurable burden of chromosomal damage that rises in proportion to the severity of their oxidative imbalance. Every patient group showed higher malondialdehyde, total oxidant status and oxidative stress index together with lower total antioxidant capacity, glutathione and superoxide dismutase than matched healthy controls, and every group also showed more structural chromosomal aberrations, micronuclei, nucleoplasmic bridges and nuclear buds. The oxidative stress index was the single biomarker most strongly correlated with cytogenetic damage, and it remained the strongest independent predictor of micronucleus frequency after adjustment for age, sex, smoking, body mass index and disease duration. The consistency of this gradient across three clinically distinct conditions supports

the view that redox imbalance is a shared upstream mechanism rather than a disease-specific epiphenomenon, which is the position taken in contemporary reviews of oxidative stress in human disease [34].

The biochemical results fit an established mechanistic framework. The elevation of malondialdehyde reflects peroxidation of membrane polyunsaturated fatty acids, the terminal stage of a radical chain reaction initiated when reactive species outstrip antioxidant buffering [5]. The reciprocal fall in reduced glutathione is biologically important because glutathione is the dominant intracellular thiol and the substrate for glutathione peroxidase and the glutathione S-transferases; its depletion leaves the cell less able to detoxify hydrogen peroxide and lipid hydroperoxides [7]. Lower superoxide dismutase activity in our patients may represent consumption of enzyme under sustained oxidant load, glycation-related inactivation, or reduced synthesis, mechanisms that have all been described in diabetic and uraemic states [21,25]. The use of total oxidant status and total antioxidant capacity, and of their ratio, gave an integrated picture that single analytes cannot provide, which is the rationale for which these automated assays were originally developed [8]. That the oxidative stress index outperformed its individual components as a correlate of chromosomal damage suggests that the balance between oxidant load and defence, rather than either term alone, determines how much damage reaches the genome.

The connection between this biochemical state and damage to chromosomes is easily understood. Hydroxyl radicals assault deoxyribose as well as the four nucleobases, leading to formation of oxidised lesions, abasic sites, single-strand breaks, and intermediate products of base excision repair with respect to single-strand breaks which may be converted into double-stranded breaks if replication occurs rapidly enough [9]. Double-strand breaks are important precursors for (i) the acentric fragments which give rise to micronuclei and (ii) the dicentric chromosomes that cause nucleoplasmic bridges to form, making it reasonable that the observed increases in micronuclei, bridges and buds at this biochemical state was due to clastogenic damage rather than solely due to aneugenic damage [13,15]. Lipid peroxidation products add a second method because malondialdehyde and 4-hydroxynonenals form exocyclic adducts that can both act as mutagens and block replication. When the amount of damage exceeds what the DNA damage response system is able to handle, misrepair and stability result [11,12], and experimental work has established that even chronic low levels of oxidant exposure creates a chromosomal instability that persists long after the exposure period [19]. Telomeres present yet another point of vulnerability because the abundant guanine repeats serve as targets for oxidation while they are repaired much less efficiently than the bulk of the chromatin, so that accelerated depletion occurs and leads to end fusion and breakage–fusion–bridge cycles and thus contributing to the number of nucleoplasmic bridges that we counted [18].

The severity of injury due to the three diseases indicates something on its own. Among chronic kidney disease patients, the oxidative stress profile was most disturbed and they revealed the greatest number of disorders and micronuclei. The correlation between the oxidative stress index and the frequency of micronuclei was in the order of $r = 0.71$. This is consistent with the historical description of oxidative stress as an unifying concept in uremia, where the accumulation of the uremic solutes, and chronic inflammation, anemia, administration of iron and depletion of the glutathione system occur together [24,25]. It was also reported that patients with end-stage renal disease have increased micronuclei and breaks in their DNA because of advanced glycation products and carbonyl stress being involved as mediators [26]. The assessment of clinical markers indicated that genomic alteration generally increases as the

kidney function diminishes, which could be one of the reasons for the high cancer incidence in patients of this group [27]. The reverse correlation between the reduced glomerular filtration rate and the frequency of micronuclei calculated for our patients supports this thesis and indicates that the cytogenetic disorder is indicating the severity of kidney failure.

In the diabetes cohort, the connection between glycated hemoglobin and micronucleus counts points towards glycemic control being a modulator of genome damage. Hyperglycemia leads to the production of mitochondrial superoxide causing activation of polyol and hexosamine pathways, protein kinase C and production of advanced glycation end-products, which is a common mechanism proposed to explain diabetic complications [20,21]. Multiple studies provide evidence of increased micronucleus blood levels in leukocytes of diabetic individuals, and the larger literature provides evidence that high levels of micronuclei are linked to obesity, diabetes and cardiovascular disorders, which all are characterized by oxidative and inflammatory processes [22]. The fact that folate supplementation is capable of reducing the level of micronuclei in diabetic patients is of particular importance, as it suggests that some level of damage can be modified, and that micronucleus readouts may be used as end-points in interventional studies [23]. The fact that duration of the disease was found to be associated with micronucleus level suggests cumulative exposure to hyperglycemic and oxidative conditions.

Hypertensive patients exhibited the least but still significant deviations from the normative values. The main source of oxidant formation in hypertension is attributed to NADPH oxidases, uncoupled endothelial nitric oxide synthase, and mitochondria, resulting in nitric oxide deficiency and endothelial dysfunction and remodelling [28]. As oxidative stress is considered to be a common cause of hypertension, including activation of the renin–angiotensin–aldosterone system [29], the lower degree of cytogenetic changes seen in this research can suggest that oxidant generation is localised in the vascular system in patients with hypertension while patients with diabetes and uraemia are exposed to systemic metabolic stress through their circulating lymphocytes. The discrepancy can be also explained via the antioxidant and anti-inflammatory properties of many classes of antihypertensive medication. Nevertheless, the increase in the number of micronuclei observed is not insignificant; it was shown that in patients with coronary artery disease elevated frequency of micronuclei correlated with an increased risk of subsequent adverse cardiac events, which demonstrates the potential prognostic value of this finding in cardiovascular medicine [30].

The clinical implications of these findings are derived from prospective data that strengthen the evidence that lymphocyte cytogenetic damage ultimately leads to disease. There are cohort studies reporting the fact that chromosomal aberrations can predict future cancer in Finnish and Italian populations without prior carcinogen exposure [16] as well as those showing that micronucleus frequency in healthy individuals can also predict future disease from data compiled in the HUMN database [17]. If chronic disease groups remain consistently at the top in these distributions, as we have suggested previously, then part of the excess cancer risk seen in patients with chronic kidney disease and diabetes may be based on oxidative damage to the genome. Oxidative stress has been shown to combine with chronic low-grade inflammation whereby immune cells continue to produce more reactive species and cytokines in a feedback mechanism directly associated with carcinogenesis [31]. Oxidative stress functions with the metabolic inflammation linking obesity and insulin resistance with cancer [32]. Ageing represents a further layer because both accumulation of damage and repair decline over time [33]. This supports ageing being an independent predictor in our regression models.

The practical value of this paper is that it suggests oxidative stress parameters, which are low-cost and can be measured on routine spectrophotometric platforms present in Iraqi hospital laboratories, can act as inexpensive proxies for genetic instability, which requires cell culture and skillful microscopy, and as a result, cannot be used for routine purposes. In an area such as Al-Samawah, which has no cytogenetic facilities, a high oxidative stress index can be used to flag patients for closer observation, or for interventions aimed at improving redox status. Such interventions may involve optimising glycaemic and blood pressure control, smoking cessation, weight loss, exercise and so on. caution needs to be taken over the matter of antioxidant supplementation since large trials involving high-doses of single antioxidant supplements have generally produced negative results, and occasionally harmful results, due to the disruption of normal physiological redox signalling (the difference between oxidative eustress and distress) [4,34].

Numerous limitations must be recognized. The cross-sectional case-control approach allows a conclusion about the association and does not demonstrate a cause-and-effect relationship between oxidative stress and chromosomal damage; reverse causation and residual confounding are possible. Although the sample size of 40 participants per group is sufficient according to the power analysis, it is limited in terms of subgroup comparisons (by sex, treatment class, and disease stage). The metabolic agents used by the patients could have had influence on redox level, including metformin, statins, and blockers of renin-angiotensin system; the study had no possibility to disentangle these effects. Antioxidants were not assessed in the diet and seasonal variation in the availability of vegetative products in Al-Muthanna region could have resulted in variations; nevertheless, the year-round recruitment of patients could help control this problem. 8-oxodG and comet assay were not conducted to directly measure the oxidative level as well as the measurement of the DNA repair capacity, which could have modified the relationship between the damage and its chromosomal expression [11]. The method of fluorescence in situ hybridization for centromere staining was not used, and hence it was impossible to separate the micronuclei of clastogenic and aneugenic origin. Lastly, the single-site recruitment decreases the generalizability of results because the patients in a public referral center may differ from other patients.

These limitations open avenues for future research. Long-term follow-up studies of the same population would show whether cytogenetic damage worsens with disease and whether it is able to predict complications or malignancy within the Iraq population. Randomized trials on interventions such as improved glycemic control, folate supplementation, or diet modification—where micronucleus count is used as an endpoint like in the previous study with diabetic patients [23]—would allow testing of the modifiability hypothesis directly. For example, using comet assay, 8-oxodG measurement, telomere length and centromeric micronucleus characterization can help explain why this happens and add patients under hemodialysis to cover the extreme of the observed spectrum. Despite the above-mentioned limitations, this study has many strengths, including matching controls properly, removing participants with major confounding exposures, blind slides' duplication with high inter-observer concordance, and simultaneous measurement of various oxidative and cytogenetic biomarkers at the level of individual participants, which is rare in the regional literature.

Conclusion

Patients with diabetes mellitus type 2, chronic kidney disease, and essential hypertension who were patients at Al-Hussein Teaching Hospital in Al-Samawah reported a significant pro-oxidant shift, displaying values that included the highest malondialdehyde, total oxidant status and oxidative stress index combined, as well as lower total antioxidant capacity, glutathione and superoxide dismutase when compared to healthy matched controls. There was also an observation of past patients having significantly more free structural chromosomal abnormalities, micronuclei, nucleoplasmic bridges, and nuclear buds as most present in chronic kidney disease secondary to diabetes mellitus and subsequently essential hypertension patients.

The redox imbalance level demonstrated the degree of cytogenetic damage: the level of oxidative stress positively correlated with micronuclei and aberration occurrences while the antioxidant factors showed negative correlation. It was also discovered that oxidative stress remained the strongest independent predictor of micronuclei occurrence after controlling such parameters as age, gender, smoking, body mass index and duration of a disease. Thus, it becomes obvious that in chronic disease oxidative stress is more than just a chemical injury.

Due to the lower cost and convenience of measuring oxidative markers compared to genetic testing, measuring oxidative stress levels can help identify chronic patients with increased risk of genetic instability. Optimizing blood sugar levels and controlling blood pressure, preserving kidney function, preventing smoking and following a diet rich in antioxidants could help manage this problem. More longitudinal studies and intervention trials using the frequency of micronuclei as an endpoint are required to establish causation and to find out if relief from oxidative stress translates into a reduced risk of developing cancer and other complications in this group of patients.

References

1. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396(10258):1204–22.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*. 2022;183:109119.
3. Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol*. 2015;4:180–3.
4. Sies H. Oxidative eustress and oxidative distress: introductory remarks. In: Sies H, editor. *Oxidative Stress: Eustress and Distress*. London: Academic Press; 2020. p. 3–12.
5. Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. 5th ed. Oxford: Oxford University Press; 2015.
6. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat Rev Mol Cell Biol*. 2020;21(7):363–83.
7. Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. *Mol Aspects Med*. 2009;30(1–2):1–12.
8. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 2005;38(12):1103–11.

9. Cadet J, Wagner JR. DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harb Perspect Biol.* 2013;5(2):a012559.
10. Valavanidis A, Vlachogianni T, Fiotakis C. 8-Hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev.* 2009;27(2):120–39.
11. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature.* 2009;461(7267):1071–8.
12. Hoeijmakers JHJ. DNA damage, aging, and cancer. *N Engl J Med.* 2009;361(15):1475–85.
13. Fenech M. Cytokinesis-block micronucleus cytome assay. *Nat Protoc.* 2007;2(5):1084–104.
14. Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutat Res.* 2003;534(1–2):65–75.
15. Fenech M, Kirsch-Volders M, Natarajan AT, Surrallés J, Crott JW, Parry J, et al. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis.* 2011;26(1):125–32.
16. Hagmar L, Bonassi S, Strömberg U, Brøgger A, Knudsen LE, Norppa H, et al. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res.* 1998;58(18):4117–21.
17. Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, et al. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis.* 2007;28(3):625–31.
18. von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci.* 2002;27(7):339–44.
19. Limoli CL, Giedzinski E, Morgan WF, Swarts SG, Jones GDD, Hyun W. Persistent oxidative stress in chromosomally unstable cells. *Cancer Res.* 2003;63(12):3107–11.
20. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature.* 2001;414(6865):813–20.
21. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res.* 2010;107(9):1058–70.
22. Andreassi MG, Barale R, Iozzo P, Picano E. The association of micronucleus frequency with obesity, diabetes and cardiovascular disease. *Mutagenesis.* 2011;26(1):77–83.
23. Dhillon VS, Thomas P, Fenech M. Effect of common polymorphisms in folate uptake and metabolism genes on frequency of micronucleated lymphocytes in a South Australian cohort. *Mutat Res.* 2009;665(1–2):1–6.
24. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int.* 2002;62(5):1524–38.
25. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol Dial Transplant.* 2003;18(7):1272–80.

26. Stopper H, Boullay F, Heidland A, Vienken J, Bahner U. Comet-assay analysis identifies genomic damage in lymphocytes of uremic patients. *Am J Kidney Dis.* 2001;38(2):296–301.
27. Schupp N, Stopper H, Heidland A. DNA damage in chronic kidney disease: evaluation of clinical biomarkers. *Oxid Med Cell Longev.* 2016;2016:3592042.
28. Touyz RM, Rios FJ, Alves-Lopes R, Neves KB, Camargo LL, Montezano AC. Oxidative stress: a unifying paradigm in hypertension. *Can J Cardiol.* 2020;36(5):659–70.
29. Griendling KK, Camargo LL, Rios FJ, Alves-Lopes R, Montezano AC, Touyz RM. Oxidative stress and hypertension. *Circ Res.* 2021;128(7):993–1020.
30. Federici C, Botto N, Manfredi S, Rizza A, Del Fiandra M, Andreassi MG. Relation between micronucleus frequency in peripheral blood lymphocytes and inflammatory markers in patients with coronary artery disease. *Mutat Res.* 2008;650(2):195–200.
31. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med.* 2010;49(11):1603–16.
32. Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. *Nature.* 2017;542(7640):177–85.
33. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. *Cell.* 2023;186(2):243–78.
34. Sies H, Berndt C, Jones DP. Oxidative stress. *Annu Rev Biochem.* 2017;86:715–48.