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Prevalence and Serum Concentrations of Aflatoxin B1 and Ochratoxin A in Healthy Adults: A Biomonitoring Study in Baghdad Province

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Abstract

Introduction: Mycotoxins are toxic secondary metabolites produced by toxigenic fungi that pose a persistent threat to global public health and food security. Aflatoxin B1 (AFB1) and ochratoxin A (OTA) are particularly notorious for their detrimental health impacts, including hepatotoxicity and nephrotoxicity. This study aimed to investigate the prevalence and serum concentrations of AFB1 and OTA among healthy adult volunteers in Baghdad Province and evaluate the community's baseline exposure to these mycotoxins in relation to demographic variables.

Methods: A cross-sectional biomonitoring study was conducted involving 96 healthy adult volunteers (40 males and 56 females; aged 15-40 years) residing in Baghdad Province. Peripheral venous blood samples were collected, and serum was isolated.

Results: Biomonitoring revealed a remarkably high prevalence of mycotoxin exposure in the study population. OTA was detected in nearly 100% of serum samples, with no significant sex differences, whereas AFB1 was detected in 42% of participants. Both OTA and AFB1 levels were significantly higher in the younger age group (≤24 years) than in those>24 years ($P=0.006$ and $P=0.009$, respectively). Furthermore, OTA exhibited a significant negative correlation with age in females, whereas AFB1 showed a significant positive correlation with age in individuals aged>24 years.

Conclusion: The widespread detection of OTA and the particularly high levels of AFB1 in serum are important for understanding the hidden impact of mycotoxin exposure in individuals from Baghdad Province. The findings highlight the urgent need for ongoing population-based biomonitoring and stringent food safety policies to address the risk associated with chronic dietary exposure contributing to cumulative renal toxicity and liver carcinogenesis.

Keywords: Aflatoxin B1, Ochratoxin A, Mycotoxins, Biomonitoring, Iraq

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Introduction

Mycotoxins, which are frequently produced secondary metabolites of numerous toxigenic fungi, pose a significant risk to food security and populations across the globe. These contaminants can induce acute or chronic disease after ingestion, inhalation and skin contact. These include extreme levels of fatigue and lethargy, diminished attention span, suboptimal immune function, and poorer cognitive ability which have been classified as common chronic sequelae attributed to mycotoxin exposure (1, 2). Moreover, chronic exposure has been related to many other debilitating injuries such as mutagenicity, teratogenicity, reproductive and endocrine impairment, cell apoptosis and myelotoxic events(3). Due to high toxicity and widespread prevalence in agricultural products, mycotoxins including aflatoxins (AFs), ochratoxin A (OTA) and zearalenone (ZEN) have aroused worldwide great public health concern (4).

When considering the different mycotoxins, aflatoxin B1 (AFB1) and OTA are among the most widely recognized for their considerable health effects as well as their high occurrence in food/feed systems around the world (5, 6). The AFs represent some of the most potent natural hepatocarcinogens, with AFB1 produced by primarily Aspergillus flavus and Aspergillus parasiticus species being the most powerful hepatotoxicity inducing agent of all. It is highly effective in producing: growth inhibition, immunotoxicity and genotoxicity; on this ground the International Agency for Research on Cancer (IARC) has classified it as a group 1 human carcinogen (7,8). Conversely, OTA, synthesized primarily by Penicillium verrucosum and Aspergillus ochraceus, exhibits profound nephrotoxic, hepatotoxic, immunotoxic, and teratogenic properties in several mammalian species (9). Consequently, the IARC has designated OTA as a Group 2B carcinogen, indicating that it is possibly carcinogenic to humans (10).

Although regulatory frameworks already exist to guarantee low contamination levels and a great amount of work is being carried out with the purpose of measure, reduce and mitigate exposure to mycotoxins, still represent a huge challenge in developing regions with environmental conditions favouring fungal growth and poor conservation measures. Both AFB1 and OTA are

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toxicologically well characterized, but epidemiologic data on human exposures from healthy adults in Iraq — one of the most important health hazards in the country — is scant. Characterizing baseline exposures to mycotoxins in asymptomatic individuals is key to generating reliable estimates of the hidden burden of long-term organ impairment arising from this specific toxin exposure and designing effective public health interventions. Accordingly, this study was conducted to determine the exposure of Healthy Adult individuals from Baghdad Province o aflatoxin B1 and OTA prevalence as well.

Materials and Methods

Ethical Considerations and Study Population

The study protocol was approved by the Ethics Committee of the College of Science Research, College of Science (Approval No. CSEC/0225/0022), on 11 February 2025. The study was conducted in accordance with the Declaration of Helsinki. All participants were provided with details of the study aim, process and risks prior to enrolment and each volunteer subsequently signed a written informed consent.

96 Subjects of healthy adult volunteers residents in Baghdad area, Iraq. At the time of enrollment, blood samples were collected from these subjects to measure circulating levels of selected mycotoxins.

Sample Collection and Processing

Peripheral venous blood from each participant was collected in sterile disposable vials without additives. The collected blood is then allowed to form a clot at room temperature for 10–20 min. The samples were then centrifuged for 20 min at 2000–3000 RPM to remove cellular elements from the serum. Serum was isolated and aliquoted into microcentrifuge tubes under clean conditions. Serum aliquots for immediate analysis (within 5 days) were stored at 2–8°C, whereas samples intended for longer storage (less than one month) were frozen upon arrival at -20°C, and sample analytes that required six months of storage were stored at −80° C to preserve analyte stability.

Analytical Methods

Serum samples were used to measure concentrations of OTA and AFB1 using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits following manufacturers' recommendations. In particular, a kit to detect OTA based on human ochratoxin A ELISA (Catalog No: ETO-2003-EU; Bioassay, China) and a competitive ELISA Kit (Cat. The results were quantified using the AFB1 commercial ELISA kit (No. E7002Hu; Elabscience, USA).

Statistical Analysis

Statistical analyses were performed to assess the association between levels of mycotoxins and demographics. Differences in medians were analysed by a non-parametric test (Mann-Whitney U-test for independent groups, e.g. for sex and age). The Spearman's correlation coefficient to study the relations between concentrations of several mycotoxins and continuous variables. The cone of correlated values was represented as a heatmap whilst the values inside the diamonds indicates correlation coefficients. In this matrix, positive correlation is represented by red hues, whereas negative correlation is represented by blue hues. A p-value of less than 0.05 was considered statistically significant for all the tests.

Results

A total of 96 blood samples were collected from adult volunteers (40 males and 56 females) aged 15–40 years. Regarding mycotoxin levels in the blood samples, 58% had undetectable aflatoxin B1 levels and 42% had detectable levels. Regarding OTA, nearly all samples exhibited detectable concentrations. OTA levels showed no significant difference between males and females (15.2 [IQR: 12.9-22.8] vs. 15.8 [IQR: 14.3-17.3] ng/mL; $p = 0.75$), while they were significantly raised in the ≤ 24 years age group compared to the > 24 years age group (17.1 [IQR: 14.9-26.0] vs. 15.4 [12.9-17.0] ng/mL; $P = 0.006$) (Figure 1). AFB1 levels were lower in males than in females, but the difference was not significant (0.35 [IQR: 0.19-0.48] vs. 0.41 [IQR: 0.26-0.50] ng/mL; $P = 0.374$). AFB1 levels were significantly higher in the ≤ 24 years age group than in the > 24 years age group (0.48 [IQR: 0.36-0.55] vs. 0.34 [0.20-0.47] ng/mL; $P = 0.009$), as shown in Figure 2.

The Spearman's rank correlation analysis heat-map matrix between OTA and AFB1 with age in normal males and females shows that only OTA showed a significant negative correlation with age in females ($P = 0.025$), as shown in Figure 3.

The heat-map matrix of Spearman's rank correlation analysis between OTA, aflatoxin B1, and age in normal individuals in the age groups ≤ 24 and > 24 years showed that only AFB1 had a significant positive correlation with age in the age group > 24 years ($P = 0.011$), as shown in Figure 4.

Discussion

Epidemiological data indicate extensive, sometimes conflicting exposure to mycotoxins in many different locations and their epidemiology continues to exhibit the complexity of a global challenge to food security and human health. In the present study, we measured serum levels of the two most relevant mycotoxins in terms of their toxicological significance: AFB1 and OTA. The frequency of exposure to mycotoxins was exceedingly high; OTA was detected in human serum samples from nearly 100% of participants but AFB1 only in 42%. The detection of these ANAs in the sera of otherwise healthy individuals advocates for repeated exposure screening to characterize populations, estimate cumulative health risk and inform evidence base public health measures and toxicological food safety policies in regions with established high-risk exposures.

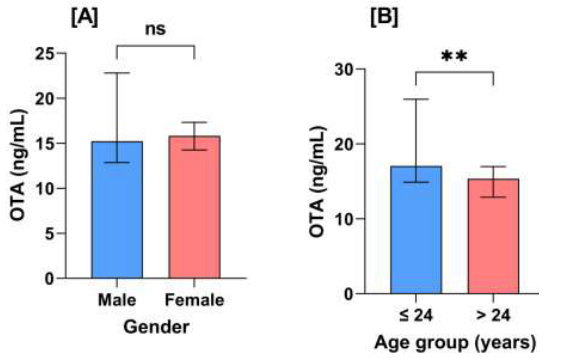

Figure 1. Serum levels of ochratoxin A in normal individuals classified by gender and age group

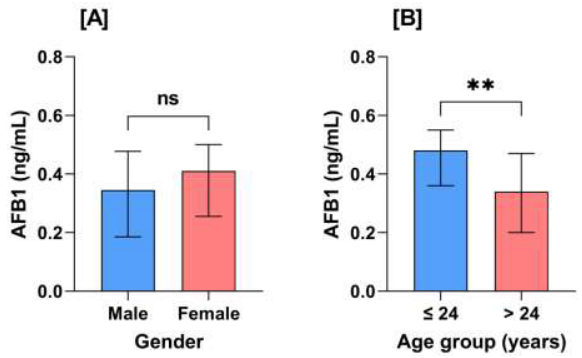

Figure 2. Serum levels of aflatoxin B1 in normal individuals classified by gender and age group

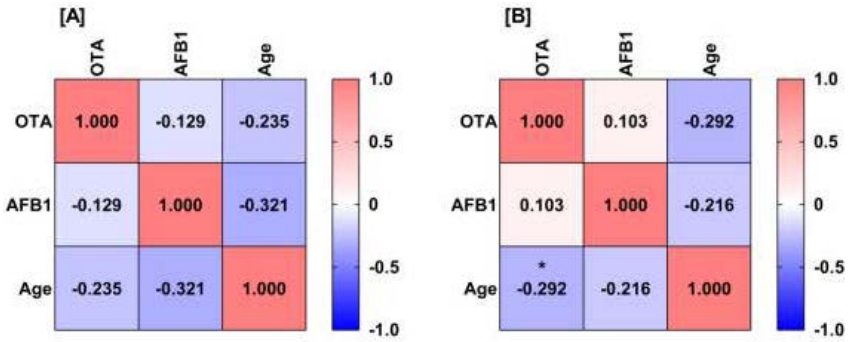

Figure 3. The Spearman's rank correlation analysis heat-map matrix between ochratoxin A, aflatoxin B1, and age in normal males [plot A] and females [plot B]

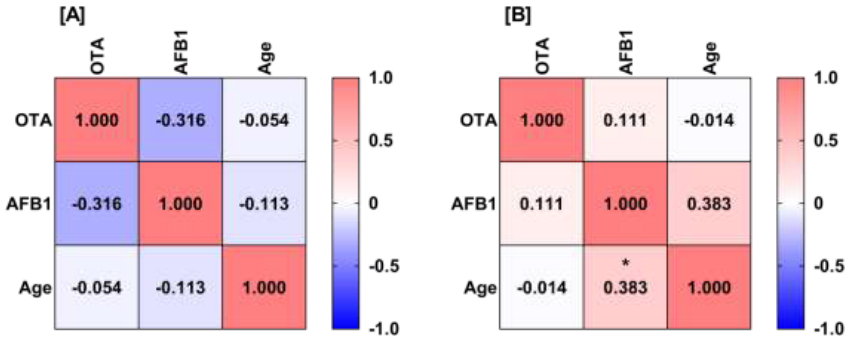

Figure 4. The Spearman's rank correlation analysis heat-map matrix between ochratoxin A, aflatoxin B1, and age in normal individuals in the age groups ≤ 24 and > 24 years [plots A and B, respectively].

The almost universal detection of OTA in the biomonitoring cohort (≥99% prevalence) is consistent with findings from international biomonitoring studies reported in the literature to date. On the other hand, a wide-ranging review by Escrivá et al., focused on mycotoxin determination in biological samples published over a decade, identified OTA as the most commonly studied mycotoxin in human biological matrices worldwide (11). This predominance has been observed owing to the peculiar toxicokinetics of OTA, which is distinct from those of other mycotoxins. Aflatoxins are rapidly metabolized in the liver and excreted quickly; whereas, OTA is distinguished by an exceptional affinity for serum albumin in rodents, as it binds loosely to plasma proteins and has a much higher concentration ratio than other naturally occurring toxins such as aflatoxins. A study found that about 99.8% of OTA in human circulation is bound to albumin, resulting in a half-life of up to 35 days. The prolonged retention time is responsible for the consistently pronounced detection rates of OTA in serum samples, despite intermittent or sporadic dietary exposure; conversely, AFB1 detection mirrors a more immediate recording of events that is attributed to its rapid hepatic metabolism (12). Baghdad residents had the highest rates of OTA detected among all nationalities screened, which is particularly significant between nations with varying dietary habits; the Middle East and North Africa region ranks as an intermediate-to high-risk area for mycotoxins since occurrence rates in MENA animal feed have been documented at 31% (13).

Our detection rate for AFB1 at 42% (Tyangmimi et al., 2023) overall, although lower than the near absence of OTA from many foods and cereals, is still a public health threat. Recent comprehensive reviews of global aflatoxin exposure have reported a wide-range of detection rates from human populations with notable

geographic distribution. European populations tend to display very low prevalence (0--1%), while areas of West Africa and Southeast Asia have near or greater than 90%. The MENA region, including Iraq has been categorized as an intermediate-to high-risk region of mycotoxin exposure (14). A meta-analysis that combined data from 323 studies covering the MENA region indicated that mycotoxins were present in 42% of animal feed, with a maximum occurrence rate found for aflatoxins (47%) and fumonisins% (13). Iraq was actually noted as a gap in the data provided in this meta-analysis, due to lack of local independent studies on mycotoxin contamination of animal feed available up until that date. The clear association between levels of contaminated food and feed sources supplied in the human serum illustrates the persistent risk of dietary exposure in Baghdad Province, whereby biomonitoring attention is warranted. The 42% detection rate for dietary AFB1 occurrence in healthy volunteers correlates well with the occurrence study of AFB1 in animal feeds (39%), suggesting that this ASE component is readily absorbed by the animal body and efficiently transmitted from animals through the food chain or directly through diet to humans.

The distinct detection patterns observed between AFB1 and OTA differ between the two compounds, likely owing to differences in their metabolic pathways and tissue distribution. AFB1 is quickly metabolised in the liver, as reported in the original study after absorption from the gastrointestinal tract. AFB1 is metabolized by the cytochrome P450 enzyme system to a highly unstable and toxic intermediate, AFB1-exo-8,9-epoxide (AFBO), that rapidly interacts with guanine residues in DNA to create AFB1-N7-guanine adducts or hydrolyzes to form AFBD-dihydrodiol. The quick metabolic activity of phenobarbital typifies why AFB1 is not present long in the circulation and thus its detection rates are more limited than OTA(15). Conversely, OTA also presents high binding affinity to serum albumin (99.8% of circulating OTA) preventing rapid clearance and allowing a longer systemic half-life. This biochemical difference may have important consequences for biomonitoring strategies, because while serum OTA levels would indicate integrated cumulative exposure throughout life, AFB1 detection will reflect recent (i.e., dietary) exposure. This is important information to keep in mind when interpreting biomarker data arising from human biomonitoring, particularly as the field rapidly develops methods that will allow analyses like this on a larger scale (16). Although the present study performed ELISA-based methods that can be easily used formass screening, LC-MS/MS technique with a better detection of individual and identificationof metabolites are more sensitive and specific(17).

Our study's greatest strength is the wealth of information on differences in mycotoxin exposure by age group. Higher levels (P 24 years). Furthermore, it was shown to be significantly and negatively correlated with age (at least in females), and AFB1 was positively associated with age above 24 years of age. This age dependence is now acknowledged as a critical factor in the assessment of mycotoxin poisoning. For example, higher levels among younger adults may reflect greater food consumption relative to body weight or different eating patterns than more established obesity or overweight populations. Li et al. performed a broad review as regards mycotoxin exposure, mentioned that when children, teenagers, and infants are considered as population subgroups with completely contrary exposure patterns to adults, new data are necessary to explain particular vulnerabilities among younger populations(17). The positive association between AFB1 and age in the elders (>24 years) and the absence of correlation between these values with BpDE are consistent with our earlier suggestion that the toxicokinetics of these compounds is complex.

In our current study, gender did not have a significant effect on OTA levels (males vs. females), and AFB1 was lower in males than in females, but this difference was not statistically significant. These demographic differences have been recognized as key determinants of mycotoxin exposure. Thenuwara et al. Additionally, the other author reviewed sex- and gender-specific considerations in mycotoxin screening and highlighted that biological (sex-based) and sociocultural (gender-based) factors significantly affect mycotoxin exposure risks and health outcomes (18). Due to physiological and hormonal variability, women may be particularly susceptible to mycotoxin effects, with possibly higher risks during sensitive periods in life (e.g., during pregnancy and lactation) (19). In contrast, men appear to have unique metabolic and immunologic reactions against these specific toxins. Socio-economic and cultural factors contribute to exposure risk as well; e.g., occupational exposure, malnutrition, and inaccessibility of healthcare based on gender. The higher urinary levels of aflatoxin M1 (AFM1) and fumonisin B1 (FB1) in women are accompanied by development of higher urinary levels of ochratoxins and type A trichothecene, which is opposite to the trends observed in males; thus, sex-hormone-related mechanisms have been implicated in some exposure routes/metabolism processing(18). The small observed sex dimorphism in our Baghdad cohort appears to explain a uniform dietary habit and economic condition in the population studied. Nevertheless, we need larger studies with more detailed exposure and dietary data to completely define sex-specific exposure patterns in Iraq.

The widespread exposure to mycotoxins has serious and multifaceted implications for the health of Baghdad's population. AFB1 and OTA are two well-characterized, potent toxins that can accumulate over time in humans. AFB1 is classified as a Group 1 human carcinogen by the IARC, estimated to account for some 14-19% of worldwide hepatocellular carcinomas (14). AFB1 is a potent carcinogen that requires metabolic activation to form an epoxide that interacts with DNA, resulting in mutations primarily at the TP53 tumor suppressor gene. This risk is particularly pronounced, associated with a>59-fold relative risk among HBV co-infected

individuals compared to apparent-unexposed, HBV-negative counterparts (14). OTA is classified as a Group 2B possible human carcinogen by the IARC and has been reported to be a major nephrotoxin (11). In particular, there is an association between chronic OTA exposure and Balkan endemic nephropathy (a progressive renal disease that is endemic to specific areas of the Balkans), and an increased risk of urinary tract tumors. Recent epidemiological literature has extended our view of the health effects associated with mycotoxins beyond cancer, showing associations with childhood stunting, immunosuppression, and increased susceptibility to infectious diseases (14, 17).

The co-occurrence of both AFB1 and OTA in the serum of healthy individuals in Baghdad Province raises significant concerns regarding synergistic toxicity, particularly with respect to hepatic and renal functions. Recent epidemiological assessments indicate that chronic low-dose exposure to multiple mycotoxins can lead to subtle but progressive organ damage, immunomodulation, and increased susceptibility to infectious diseases (14,18). In population with restricted health services access, poor nutrition and a prevalence to infectious disease like hepatitis B and C, the cumulative burden of mycotoxins on health is especially alarming; a study by Ismail et al. indicated that Baghdad contributes 27% of dialysis patients, which signifies the burden of end-stage kidney failure in Iraq (20). Although this study did not specifically investigate mycotoxin exposure as a contributing factor, the high prevalence of chronic kidney disease in the region suggests that environmental toxins, including mycotoxins, may contribute to the disease etiology. The potential synergistic effects of AFB1 and OTA on renal function require further investigation.

Understanding the sources of mycotoxin exposure in the Baghdad population requires consideration of global patterns of food contamination. Li et al. systematically examined mycotoxin contamination patterns across major food categories (17). Cereals and cereal products are major sources of mycotoxin exposure, with maize contaminated with AFBs, fumonisins, trichothecenes, and zearalenone; wheat contaminated with trichothecenes, DON, OTA, and other toxins; and rice primarily contaminated with ZEA. In many countries, AFM1 (the principal metabolite of AFB1) is legally monitored in milk for animal-derived products and aflatoxins are documented to be stable throughout milk processing. In the review, they noted that an estimated 60-80% of global crops are contaminated with mycotoxins, and 20% exceed European Union legal limits. For this part of the world, cereals are the major route of exposure for most of population; high rate of incidence and prevalence beings probably related to the high thats mycotoxin contamination in grain supplies explaining similarities in serum concentrations we observed our study with those ones Aaron B. As the data on staple dietary products of Iraq documented in this study indicate that wheat and rice are likely to be the major contributors to AFB1 and OTA exposure (21-24).

This study has important implication for continuing public health policy and interventions in Iraq. In fact, the exceptionally high prevalence of mycotoxins at PH level (e.g. highest concentrations of OTA ever reported in healthy volunteers and even higher concentrations of AFB1) implies that these agents are an ongoing threat because they repeatedly contaminate our food supplies. Li et al. reported that an in-depth examination of inequalities in community-level exposure is crucial for targeting interventions(17). Communication, appropriate medication therapeutic courses need to accompany targeted susceptible populations including pregnant women, linemen, children and adults with Arrhenius immunocompromised systems or a pre-volution fly by way of liver or rift disease. Appropriate measures should probably be preventive and more youth-focused, given the age differences observed in this study. Therefore, the association between mycotoxin exposure and chronic kidney disease in Iraq needs to be further investigated by determining if a correlation exists between the severity of mycotoxin levels and patients' clinical outcomes. Instituted public health interventions should address poor practices in food storage and handling that are likely to lead to fungal contamination, lax enforcement of hygiene legislation, and inadequate monitoring of food components, thereby predisposing to mealybug-associated degradation.

Conclusion

The presence of AFB1 and OTA in the serum of healthy adults in Baghdad Province indicates a long-term exposure to these mycotoxins, which may be an alarming public health issue in Iraq. However, the very high prevalence of OTA- and AFB1-positive samples in nearly half of the subjects corresponds to the intermediate- and high-risk profiles observed for the MENA region in recently published international data. The findings of age- and sex-specific differences in exposure levels underscore the need to account for demographic factors in mycotoxin risk assessment. This study provides novel data from a hitherto understudied region and highlights the need for improved food safety regulations, ongoing biomonitoring programs, and targeted public health interventions to mitigate exposure to mycotoxigenic fungi in sandwiches in Iraq. Because the cumulative health outcomes of chronic mycotoxin exposure in humans, and, more importantly, the synergistic toxicities of AFB1 and OTA on hepatic and renal functions, require prompt action by public health authorities, an uninterrupted national plan to coordinate multisectoral action is urged for the risk management of mycotoxins in the country.

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We acknowledge the participation of all study participants. Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contribution

Conceptualization: Faheema Jabbar, Sinai Waleed Mohammed, Ahmad Yosif Hanoon
Data Curation: Ahmad Yosif Hanoon
Formal Analysis: Ahmad Yosif Hanoon
Funding Acquisition: Faheema Jabbar, Sinai Waleed Mohammed, Ahmad Yosif Hanoon
Investigation: Faheema Jabbar
Methodology: Sinai Waleed Mohammed
Project Administration: Sinai Waleed Mohammed, Ahmad Yosif Hanoon
Resources: Sinai Waleed Mohammed, Ahmad Yosif Hanoon
Software: Faheema Jabbar, Sinai Waleed Mohammed
Supervision: Sinai Waleed Mohammed
Validation: Faheema Jabbar, Sinai Waleed Mohammed
Visualization: Faheema Jabbar, Sinai Waleed Mohammed
Writing – Original Draft: Faheema Jabbar, Sinai Waleed Mohammed
Writing – Review & Editing: Faheema Jabbar, Sinai Waleed Mohammed

Competing Interests

There are no conflicts of interest.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

This study was approved by the ethical committee of the College of Science Research on February 11, 2025 (CSEC/0225/0022). All participants gave informed consent and volunteered to donate blood during recruitment.

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