



Assessment of blood mercury, immune response, heat shock and oxidative stress marker in free-ranging chicken (*Gallus Gallus domesticus*) from gold mining areas in Zamfara State, Nigeria

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ABSTRACT

We investigated serum mercury, immune response, heat shock and oxidative stress marker in free-ranging chicken (*Gallus Gallus domesticus*) from Artisanal and small-scale gold mining (ASGM) areas. Six groups were created from sixty free-ranging chickens (*Gallus Gallus domesticus*), three each from ASGM and non-ASGM areas. Immune response, heat shock and oxidative stress markers were analysed, and multivariate and univariate data analysis were used to discriminate the difference in the study group. Results revealed a significant increase in serum mercury (Hg) from chicken in ASGM areas. Superoxide dismutase, glutathione peroxidase (GPx), glutathione (GSH), catalase and total antioxidant capacity significantly decreased, while lactic acid dehydrogenase and malondialdehyde (MDA) content significantly increased in animals from ASGM areas. A unit increase in GPx and GSH significantly corresponds to a decrease in serum Hg by 0.001 $\mu\text{g/mL}$ and 0.206 $\mu\text{g/mL}$, respectively. While a unit increase in MDA significantly corresponds to an increase in serum Hg level by 0.003 $\mu\text{g/mL}$. The activities of HSP-70, TNF- α , IL-1 β and IL-6 were significantly higher in the serum of chicken from ASGM areas. All significance at $p < 0.05$. ASGM activities result in mercury bioaccumulation with potential health effects caused by dysregulated immunity, heat shock and oxidative stress.

Introduction

Mercury is a vast environmental toxicant. Therefore, humans and animals are usually exposed to their various types (Adewumi. and Laniyan, 2020; Adewumi and Laniyan, 2020; Ali et al., 2019; Esdaile and Chalker, 2018; Matsumoto and Liu, 2020; Yang et al., 2020). Mercury exposure has been reported to occur through food intake, dental amalgam outgassing, vaccines that contain preservatives like thiomersal, drinking water, anthropological and occupational exposure like gold mining, industrial and agricultural products (Bank, 2020; El Mahmoud-Hamed et al., 2019; Esdaile and Chalker, 2018; Tibau and Grube, 2019; UNEP, 2019). Mercury has been reported in tissues of free-grazing animals in gold mining areas globally (Aendo et al., 2022; Binkowski et al., 2021; Yin et al., 2017). This constitutes a grave health challenge to animals, especially free-ranging chickens in gold mining areas (Lee et al., 2020; Zhou et al., 2020).

Zamfara is rich in natural resources, and in particular gold reserves which was discovered in some parts of the state such as Maru, Anka, and Bukkuyum Local Government Areas (MMSD, 2017). These gold reserves have been around for decades and are currently excavated by artisanal and small-scale gold miners (ASGMs). One of the crucial sources of livelihood in the mining areas is ASGM, but it also comes with hazards resulting from the use of poisonous mercury by miners to get the gold out of the ore. Thus, they are constantly exposed to mercury (Afrifa et al., 2019; Bank, 2020; Esdaile and Chalker, 2018; Mason et al., 2019; UNEP, 2019).

Anthropogenic (human-caused) activities such as mercury-dependent ASGM is one of the major sources of mercury emissions on the planet (Esdaile and Chalker, 2018; UNEP, 2019). In this procedure, elemental mercury is utilised as an amalgam to extract gold from ore. Hand isolation of the amalgam is common, followed by heating to distil the mercury and remove the gold (often with a torch or over a stove). ASGM emits over 1000 tonnes of mercury yearly via tailings

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and vaporised mercury. The miners' health is jeopardised, as breathed mercury results in health challenges. Mercury poisoning of water and soil, as well as subsequent accumulation in food staples, a key source of dietary protein in many ASGM regions impact on the health of animals (including free-range chickens) living near these mining areas (Adewumi and Laniyan, 2020; Aendo et al., 2022; Binkowski et al., 2021; Esdaile and Chalker, 2018; Giri and Singh, 2019; UNEP, 2019; Yin et al., 2017). Since earlier researchers have shown that an enormous amount of methylmercury (MeHg) in food chains might be accumulated in mammals in the environment. Consequently, birds have been reported as biological indicators of environmental Hg contamination (Aendo et al., 2022; Binkowski et al., 2021; Yin et al., 2017).

Immunotoxicity studies have linked exposure to mercury with the generation of reactive oxygen species (ROS) production and induction of stress biomarkers such as heat shock proteins (HSP), especially HSP70 (Balali-Mood et al., 2021; Björklund et al., 2020; da Cunha Bataglioli et al., 2019; Fan et al., 2020; Han et al., 2021; Han, Biqi et al., 2021; Renu et al., 2021; Yang et al., 2020). Mercury contamination perturbed expression of proteins that perform important cellular activities like catalytic action, binding, cell division, localisation, cellular regulation, metabolic processes, and development processes; thus, its toxicity.

Since there is a dearth of information on immune response, oxidative and heat stress response in free-ranging chicken from gold mining areas in Nigeria, this study is designed to investigate immune response, oxidative and heat stress parameters in free-ranging chicken (*Gallus Gallus domesticus*) from gold mining areas in Anka LG and compared with those in non-gold mining areas (Gusau LG). This work will provide scientific basis for exploring the extent of cell damage in exposed animals due to gold mining activities that might increase mercury in the environment to find reliable and long-lasting remediation actions.

Methodology

Ethical approval

The Committee on Animal Use and Care, Ahmadu Bello University, Zaria, reviewed and approved this study with Approval No: ABU-CAUC/2021/059. The study also followed the National Institutes of Health's recommendations for the care and use of laboratory animals, and adequate procedures were made to ensure that the animals were not subjected to excessive pain and discomfort during the study.

Study area and sample collection

Free-ranging adult chickens (*Gallus Gallus domesticus*) were the targeted species for the study. They were randomly selected in the three polluted areas (Abare [5°57'24.95"E: 12° 4'37.79"N], Dareta [5°57'17.49"E: 12° 1'49.80"N] and Sunke [5°54'44.14"E: 11°53'45.56"N]) in Anka LG (Fig. 1) (evidence of recent ASGM activities as Nigeria government has banned ASGM activities in Zamfara state) and unpolluted areas (Tashar Magami [6°40'7.38"E: 12° 9'28.63"N], Tudun Wada [6°44'27.24"E: 12°9'7.55"N] and Ungwar Yarima [6°39'47.02"E: 12°11'8.47"N]) in Gusau LG. In this cross sectional study, we randomly selected ten (10) adult chickens with bird trap from about 50 adults available during sampling in each study area, thus ten (10) samples from each location (maximum of 1km radius from the GPS coordinates shown above). Mercury was the target for this study as previous reports have shown that mercury amalgam is used for gold processing by ASGM in Zamfara state Nigeria (Adewumi and Laniyan, 2020; Johnbull et al., 2018; Uriah et al., 2013). ASGM activities have thus contributed to increased mercury concentration in Zamfara (Anka LG) gold mining areas. Similar reports of elevated mercury in gold-mining areas due to ASGM activities have been shown elsewhere, too (Afrifa et al., 2019; Bank, 2020; Esdaile and Chalker, 2018; Mason et al., 2019).

Mercury analysis

One millilitre of whole blood was dissolved in 5 mL HNO₃ (nitric acid). This was then followed by digestion in a 1600 W microwave at 200 °C for 15 min before being kept in the oven for another 15 min. The mixture was then added to 2 mL H₂O₂ (hydrogen peroxide) solution. The mixtures were maintained in the tubes for sixty minutes. The mixture was made up to 10 mL with distilled water in polypropylene flasks and maintained at 4° Celsius. Cold vapour atomic absorption spectrometry (CVAAS) was used to determine Hg amounts in whole blood (Yilmaz et al., 2015).

Pro-inflammatory cytokines and heat shock protein 70

The serum concentrations of Interleukin 1 beta (IL-1 β), Interleukin 6 (IL-6), tumour necrosis factor (TNF- α) and Heat Shock protein 70 (HSP70) were determined using commercial chicken Enzyme-linked immunosorbent assay (ELISA) kits (Adlitteram Diagnostic Laboratories, San Diego, CA, USA). All ELISA were performed with Molecular Devices SpectraMax Plus 384 Microplate Reader (Sunnyvale, CA).

Oxidative stress marker

Serum sample was used to evaluate the activities of oxidative stress indicators which were superoxide dismutase (SOD), glutathione peroxidase (GPx), Malondialdehyde (MDA), catalase (CAT), glutathione (GSH) and the concentration of lactic acid dehydrogenase (LDH) with the kits according to the manufacturer's instructions (Nanjing Jiancheng Bio-engineering Institute, Nanjing, China). GSH content was expressed as μ M/L. All analysis were performed with Molecular Devices SpectraMax Plus 384 Microplate Reader (Sunnyvale, CA).

Total antioxidant capacity (TAC)

The serum total antioxidant capacity (TAC) activities were determined using a previously reported methodology (González et al., 2020; Jimoh, 2019; Koracevic et al., 2001). The reactive reagent contain 0.5 mL of 10 mmol/L Sodium-Benzozate, 0.2 mL of H₂O₂ of 10 mmol/L, 0.49 mL of 100 mmol/L, pH = 7.4 phosphate buffer and 0.2 mL of 2 mmol/L Fe-EDTA complex and 2 mmol/L ammonium ferrous sulfate then left at 25°C for 60 min.

Statistical analysis of data

Principal components analysis (PCA) and hierarchical clustering (heatmap) were used to cluster data into homogeneous groups based on mercury level and oxidative stress parameter. One-way analysis of variance (ANOVA) and Tukey's post hoc were used to determine significant difference. Robust logistic regression was used to evaluate the associations between body weight, study location, oxidative stress markers and serum mercury levels. $p < 0.05$ was considered significant. SPSS 24.0 (SPSS, Chicago, IL, USA) was used for statistical analysis.

Results

Chickens from Gusau metropolis (Tasha Magami, Unguwar Yarima and Tudunwada) and those from gold mining areas (Abare, Dareta and Sunke) served as control and those with potential mercury exposure, respectively. The Shapiro-Wilk test shows that data generated for this study was normally distributed ($p > 0.05$). The body weight of chicken used in the study ranged from 1028.11700 $\pm$ 171.9 g to 891.71030 $\pm$ 198.7 g recorded in animals sampled from Abare and Sunke, respectively. However, there is no significant difference in the body weight of animals from different locations used in the study. The mercury level in animals from gold mining areas are statistically similar and ranged from 0.08 to 0.09 μ g/L while it was not detected in animals from Gusau L.G.

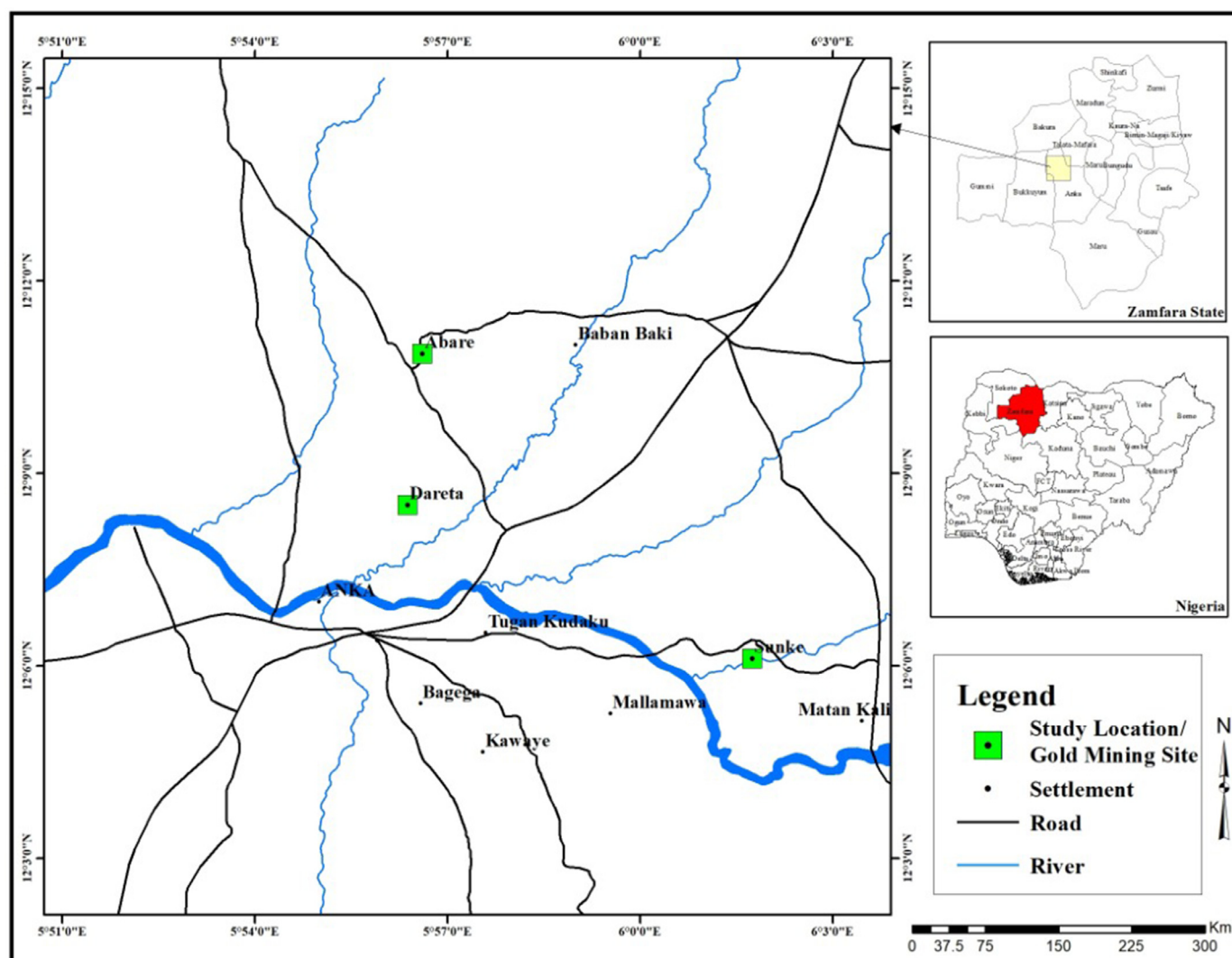


Fig. 1. Location of gold mining areas (Abare, Dareta and Sunke villages) of Anka Local Government Area, Zamfara State, Northwest Nigeria.

Multivariate analysis to distinguish Heat shock protein, pro-inflammatory cytokines and Oxidative stress marker

Unsupervised multivariate analysis called principal component analysis (PCA) was employed to analyse whether it was possible to differentiate control (Tasha Magami, Unguwar Yarima, Tudunwada) from animals sampled from gold mining areas (Abare, Dareta and Sunke) on the basis of Heat shock protein, pro-inflammatory cytokines and Oxidative stress marker. PCA score plot showed that control group could be distinguished from animals in gold mining areas (Fig. 2A, B). Hierarchical clustering Heatmaps which provides intuitive visualisation of the data was used to corroborate ANOVA result. Each coloured cell on the map corresponds to a concentration value of each Heat shock protein, pro-inflammatory cytokines and oxidative stress marker with samples in rows and features in columns (Fig. 2C, D).

Heat shock protein and pro-inflammatory cytokines

As shown in Table 1, there is no significant difference in the mean serum HSP-70, TNF- α , IL-1 β and IL-6 in animals from Tasha Magami, Unguwar Yarima and Tudunwada, as similar results were obtained from animals in gold mining areas (Abare, Dareta and Sunke). The activities of HSP-70, TNF- α , IL-1 β and IL-6 were significantly (< 0.01) higher in the serum of mercury-exposed chicken from mining areas when compared

with those assessed in the control group (Table 1). Furthermore, HSP-70, TNF- α , IL-1 β and IL-6 are significant correlate ($p < 0.01$) of Hg levels from the study group (Table 2).

As shown in Table 3, both body weight and gender did not play significant roles in the variation of serum Hg level while location significantly affected Hg levels in the study group as seen in unstandardized coefficient (0.026), 95% confidence interval (0.022; 0.030) and significant p-value ($p < 0.01$). HSP-70, TNF- α , IL-1 β and IL-6 were not significant predictors of serum mercury level in the local chicken show that they are not significant ($p > 0.05$) predictor of serum Hg.

Oxidative stress marker

As shown in Table 1, there is no significant difference in the serum SOD, GPx, LDH, MDA, GSH, Catalase and TAC in animals from Tasha Magami, Unguwar Yarima and Tudunwada. Also, a similar result was obtained from animals in gold mining areas (Abare, Dareta and Sunke). The activities of these oxidative stress markers (SOD, GPx, GSH, Catalase and TAC) were significantly ($p < 0.01$) lower in the serum of mercury-exposed chicken from mining areas when compared with those assessed in the control group (Table 1). However, LDH and MDA were significantly elevated in the serum of chickens from Hg polluted areas. Furthermore, SOD, GPx, LDH, MDA, GSH, CAT and TAC significantly correlate with Hg levels in the study group (Table 2).

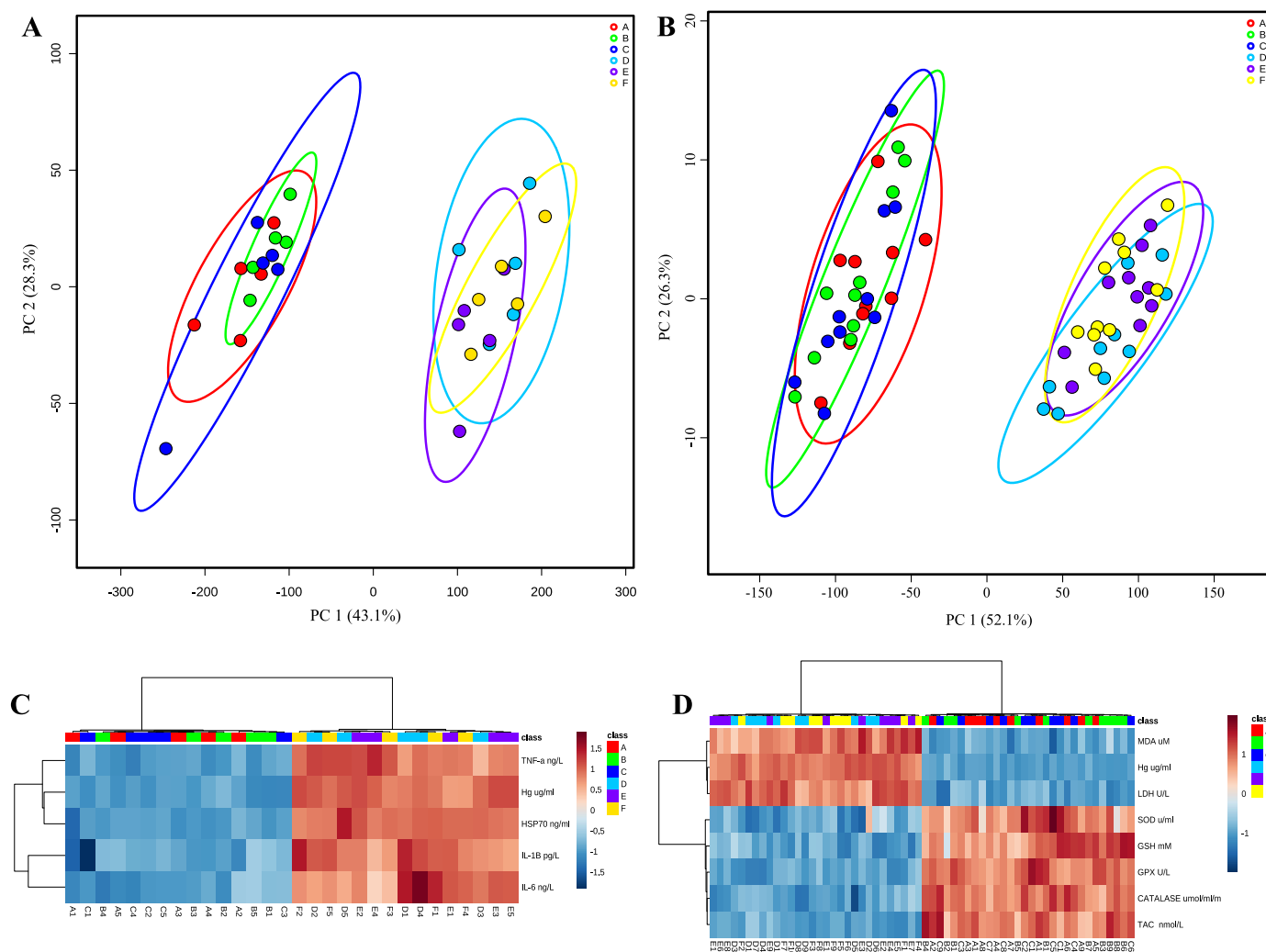


Fig. 2. Principal component analysis (PCA) developed from Heat shock protein and pro-inflammatory cytokines (A) and Oxidative stress marker (B) of control and animals from gold mining areas. Unsupervised hierarchical clustering shown as heatmap of Heat shock protein and pro-inflammatory cytokines (C) and Oxidative stress marker (D) of control and animals from gold mining areas. Red and blue indicate increased and decreased levels, respectively. Control (A; Tasha Magami, B; Unguwar Yarima and C; Tudunwada) and animals from gold mining areas (D; Abare, E; Dareta and F; Sunke).

Multiple regression analyses oxidative stress marker (SOD, GPx, LDH, MDA, GSH, Catalase and TAC) as a predictive factor for serum mercury level in local chicken showed that a unit increase in GPx and GSH significantly ($p < 0.05$) correspond to a decrease in serum Hg level by $0.001 \mu\text{g/mL}$ and $0.206 \mu\text{g/mL}$, respectively (Table 3). While a unit increase in MDA significantly ($p < 0.05$) corresponds to an increase in serum Hg level by $0.003 \mu\text{g/mL}$. But SOD, LDH, Catalase and TAC were not significant predictors of serum Hg.

Discussion

Significantly higher serum mercury levels in chickens from gold mining areas reported in this study agrees with earlier studies that reported high mercury in organs and blood from free-grazing duck in Northern Thailand biggest gold mining area, poultry in the Wanshan Mine, China and along the coast of the English Channel (Aendo et al., 2022; Binkowski et al., 2021; Yin et al., 2017). This is as a result of anthropogenic activities of mercury-dependent ASGM that usually use Hg to purify gold (Esdaile and Chalker, 2018; UNEP, 2019). ASGM activity thus emits tonnes of mercury into the ecosystem and food chain.

Several studies have shown that mercury-induced toxicities and bioaccumulation results in pronounced perturbation in various

metabolic and physiological pathways in microorganisms and animal models (Balali-Mood et al., 2021; Giri and Singh, 2019; Guzzi et al., 2021; Moniruzzaman et al., 2021; Olsvik et al., 2021; Seco et al., 2020; Slaveykova et al., 2021; Yang et al., 2020; Zhou et al., 2020). Oxidative stress from increased free radical formation and perturbed antioxidant system has been implicated in many cellular stressors in chickens (Saracila et al., 2021; Surai et al., 2019). Consequently, ROS is generated in excess, leading to the overwhelmed antioxidant defense system as the activity of antioxidant proteins (CAT, SOD, GSH and GPx) decrease in chicken. Furthermore, elevated MDA levels are caused by overexpression ROS and blocking the activities of both SOD and CAT. Earlier reports have shown a direct relationship between heavy metal exposure and enhanced lipid peroxidation, suggesting that MDA could be a valuable stress biomarker. El-Gendy et al. (2020) and Saracila et al. (2021) have implicated elevated MDA from increased ROS as a likely cause of increased DNA damage. However, complete data on the oxidative stress induced by mercury bioaccumulation in free-ranging chicken in Zamfara gold mining areas is scarce.

One of the most prominent origin of mercury emissions on planet earth is through anthropogenic (human-caused) activities in the form of mercury-dependent ASGM (Esdaile and Chalker, 2018; UNEP, 2019). As an amalgam, elemental mercury is employed to isolate gold from ore

Table 1

Variation in heat shock protein, inflammatory cytokines and oxidative stress marker due to Hg exposure

Parameter	TashaMagami	UnguwarYarima	Tudunwada	Abare	Dareta	Sunke
HSP70 (ng/mL)	2.697 ± 0.416 ^a	2.790 ± 0.109 ^a	2.895 ± 0.038 ^a	6.195 ± 0.413 ^b	6.020 ± 0.170 ^b	5.984 ± 0.154 ^b
TNF- α (ng/L)	153.324 ± 12.878 ^a	155.338 ± 7.111 ^a	158.351 ± 12.959 ^a	293.983 ± 24.076 ^b	303.449 ± 18.406 ^b	301.231 ± 10.370 ^b
IL-1 β (pg/L)	1062.981 ± 39.234 ^a	1100.770 ± 26.789 ^a	1067.193 ± 65.696 ^a	1336.984 ± 34.955 ^b	1296.563 ± 30.283 ^b	1334.964 ± 40.520 ^b
IL-6 (ng/L)	12.499 ± 0.442 ^a	12.5347 ± 0.556 ^a	12.412 ± 0.333 ^a	16.116 ± 0.971 ^b	15.726 ± 0.659 ^b	15.743 ± 0.660 ^b
SOD (u/ml)	2.897 ± 0.111 ^a	2.854 ± 0.135 ^a	2.976 ± 0.154 ^a	2.346 ± 0.187 ^b	2.373 ± 0.116 ^b	2.332 ± 0.073 ^b
GPx (U/L)	99.524 ± 3.906 ^a	101.242 ± 3.042 ^a	100.157 ± 4.147 ^a	72.919 ± 2.344 ^b	74.251 ± 2.458 ^b	75.550 ± 2.617 ^b
LDH (U/L)	380.939 ± 19.980 ^a	372.414 ± 24.565 ^a	371.528 ± 22.704 ^a	535.151 ± 29.147 ^b	547.914 ± 21.422 ^b	541.610 ± 18.999 ^b
MDA (uM)	14.274 ± 0.846 ^a	14.421 ± 0.663 ^a	13.751 ± 0.668 ^a	19.749 ± 1.019 ^b	20.215 ± 1.061 ^b	20.507 ± 1.007 ^b
GSH (mM)	0.385 ± 0.016 ^a	0.384 ± 0.021 ^a	0.390 ± 0.013 ^a	0.317 ± 0.011 ^b	0.313 ± 0.010 ^b	0.314 ± 0.010 ^b
C (umol/ml/min)	21.895 ± 0.969 ^a	21.818 ± 0.858 ^a	22.507 ± 1.066 ^a	14.819 ± 1.292 ^b	15.259 ± 0.962 ^b	15.308 ± 0.613 ^b
TAC (nmol/L)	2.176 ± 0.088 ^a	2.161 ± 0.142 ^a	2.178 ± 0.091 ^a	1.623 ± 0.057 ^b	1.599 ± 0.050 ^b	1.571 ± 0.042 ^b

Heat shock protein 70 (HSP 70), Tumour necrosis factor alpha (TNF- α), Interleukin 1 beta (IL-1 β), Interleukin 6 (IL-6). Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Malondialdehyde (MDA), Lactic acid dehydrogenase (LDH) and Glutathione (GSH), Catalase (C) and Total antioxidant capacity (TAC).

Data are presented as mean ± standard deviation, Different superscript in a row are significantly different at $p < 0.05$.

Table 2

Correlations between serum mercury level in local chicken with heat shock protein, inflammatory cytokines and oxidative stress.

Parameter	Pearson's (r)
HSP70 ng/ml	0.977**
TNF- α ng/L	0.973**
IL-1 β pg/L	0.933**
IL-6 ng/L	0.923**
SOD (u/ml)	-0.897**
GPx (U/L)	-0.968**
LDH (U/L)	0.954**
MDA (uM)	0.949**
GSH (mM)	-0.94**
C (umol/ml/min)	-0.955**
TAC (nmol/L)	-0.958**

Note: All correlations reported are two-tailed.

** Significant at $p < 0.01$.

Heat shock protein 70 (HSP 70), Tumour necrosis factor alpha (TNF- α), Interleukin 1 beta (IL-1 β), Interleukin 6 (IL-6). Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Malondialdehyde (MDA), Lactic acid dehydrogenase (LDH) and Glutathione (GSH), Catalase (C) and Total antioxidant capacity (TAC).

in this process. The amalgam is typically extracted manually by hand and subsequently exposed to heat to distil the mercury and remove the gold (often with a torch or over a stove). Each year, ASGM releases over 1000 tonnes of mercury from tailings and vaporised mercury. The miners' health is jeopardised, with inhaled mercury causing health issues. Mercury pollution of soil and water, as well as subsequent aggregation in food staples—a significant source of dietary protein in many ASGM regions—affects the animals (including free ranging chickens) around these mining sites (Adewumi and Laniyan, 2020; Esdaile and Chalker, 2018; Giri and Singh, 2019; UNEP, 2019).

Studies have shown that mercury related cell death primarily require the generation of reactive oxygen species (ROS) and proteins associated with mercury toxicity, and are involved in cellular regulation, binding catalytic activity, cell division, localization, development and metabolic processes (da Cunha Bataglioli et al., 2019; Yang et al., 2020). Thus the reduced antioxidant reported from gold mining sites reported in this study.

Hg toxicity in gold mining regions may ensue due to its ability to destroy cells through inhibition of essential macromolecules like polynucleotides and enzymes. Hg binding on the outer membrane has an impact on ion transport and cell membrane rupture (Yang et al., 2020). Mercury has a strong affinity for sulfhydryl (-SH) and phosphate groups in reaction. Mercury influences antioxidant potentials through regulation of both enzymatic antioxidants (ascorbate peroxidase, superox-

Table 3Regression analysis of HSP-70, TNF- α , IL-1 β , IL-6 and oxidative stress as a predictive factor for serum mercury level in local chicken.

Description	β	95% CI*	p
Body weight (g)	-0.00002061	(0)	0.638
Location	0.026	0.022; 0.030	< 0.01
Gender	-0.001	-0.027; 0.026	0.971
HSP70 ng/ml	0.014	-0.001; 0.029	0.064
TNF- α ng/L	0	0; 0.001	0.028
IL-1 β pg/L	0.0000399	0	0.422
IL-6 ng/L	0.001	-0.006; 0.008	0.777
SOD (u/ml)	.000	-0.018; 0.019	0.962
GPx (U/L)	-0.001	-0.002; -0.001	0.000
LDH (U/L)	0.00008764	0.000	0.054
MDA (uM)	0.003	0.000; 0.005	0.023
GSH (mM)	-0.206	-0.376; 0.035	0.019
C (umol/ml/min)	-0.002	-0.004; 0.001	0.153
TAC (nmol/L)	-0.016	-0.046; 0.014	0.277

β : Unstandardized coefficient represents the amount of change in a predictive variable due to a unit change of Hg level. Confidence interval (CI).

Heat shock protein 70 (HSP 70), Tumour necrosis factor alpha (TNF- α), Interleukin 1 beta (IL-1 β), Interleukin 6 (IL-6). Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Malondialdehyde (MDA), Lactic acid dehydrogenase (LDH) and Glutathione (GSH), Catalase (C) and Total antioxidant capacity (TAC).

Significant at $p < 0.05$.

ide dismutase and glutathione reductase) as well as non-enzymatic antioxidants (non-protein thiols and glutathione). Sulfhydryl groups in enzymes and other thiol-containing compounds like cysteine and glutathione bond to inorganic forms of mercury (Behzadfar et al., 2020; Kumari et al., 2020). Glutathione (GSH) is non-protein thiol that modulate signalling, redox equilibrium, controlling gene expression and transcription factors, nuclear pathways/processes like epigenetic mechanisms in avian (Surai, 2020; Surai et al., 2021). Thus reduced GSH activity reported in the chickens from gold mining areas point to redox imbalance which might culminate into oxidative stress in the animals from this group. This is similar to earlier reports (Behzadfar et al., 2020; Yang et al., 2020). Recent literatures also support this by suggesting that methyl mercury can bring about increased production of ROS that may either diminish the level of GSH or trigger an adaptive response to oxidative stress by elevating the level of GSH (Budnik and Casteleyn, 2019; Yang et al., 2020). From above, it is important to reiterate that protective effects are induced by intracellular low-molecular-weight thiol glutathione (GSH), that is important as a cofactor for glutathione peroxidase (a selenoenzyme). GSH production capability is strong in astrocytes, particularly in cortical astrocytes of brain cells, which may ex-

plain their greater resistance to mercury poisoning. (Bjørklund et al., 2017).

Both antioxidant enzymes activities and oxidative damage to macromolecules due to potential mercury exposure in ASGM areas were measured in our research to assess oxidative stress (DNA, proteins and lipids) from free ranging chicken in gold mining (Abare, Dareta and Sunke) and non-gold mining (TashaMagami, Unguwar Yarima and Tudunwada) areas of Zamfara state, Nigeria. There was a significant decline in the activity of antioxidants (SOD, GPx, GSH and CAT) in the serum of chickens gathered from the mining areas, compared to those from the reference site (Gusau). This is similar to earlier reports where exposure to mercury significantly decreased superoxide dismutase (SOD) activity and GSH level in mice (Li et al., 2019, 2021; Zhao et al., 2021). Our result is also in line with other studies that reported Hg exposure could reduce CAT, SOD, GSH and GPx in chicken, Seabird (Shearwaters), Ruddy ducks *Oxyura jamaicensis* and mallards *Anas platyrhynchos* (Fan et al., 2020; Hoffman and Heinz, 1998; Hoffman et al., 1998; Ma et al., 2018; Soldatini et al., 2020), thus oxidative stress (Chu et al., 2020; Surai et al., 2021). Reduced antioxidant enzyme activity have also been reported in animals exposed to varying environmental stressors, especially heavy metals (El-Gendy et al., 2020; Yousef et al., 2019). In addition, CH_3Hg^+ and Hg^{2+} (organic and inorganic mercury) can attach to membrane phospholipids and proteins, inhibit Na^+ and K^+ dependent ATPases, stop trans-membrane amino-acid transport, inhibit enzyme activity, and improve lipid peroxidation, all of which can lead to oxidative DNA damage and cell death (Bjørklund et al., 2017; Novo et al., 2021; Raj and Maiti, 2019).

This finding may be interpreted in the following way: elevated concentrations of mercury in the cell due to mining activity might induce ROS generation and destruction of plasmatic membranes (Chung et al., 2019; Fujimura and Usuki, 2020; Li et al., 2021; Novo et al., 2021). This might be due to the fact that mercury or its compounds like MeHg is capable of inducing peroxidation due to its interaction with polyunsaturated fatty acids that affect mitochondrial homeostasis which culminate in the increase of ROS production (Novo et al., 2021; Wagner et al., 2010). Another reason for this is that oxidative damage affects Mitochondrial DNA than nuclear DNA, presumably because mitochondria are the primary sources of ROS, but its DNA repair mechanisms: mitochondrial fusion, fission, and mitophagy, which serve as quality control events for cleaning damaged mtDNA, are slightly limited (Balali-Mood et al., 2021; Rong et al., 2021). Consequently, continuous oxidative stress damage to mitochondria, in particular, is bound to compromise mitochondrial structure and result in mitochondrial malfunction. This culminate in release of more ROS by prolonging the cell cycle as against antioxidant and DNA damage (Li et al., 2021).

Significant increase in MDA level in chicken from gold mining areas is similar with other reports in mice and chicken (Fan et al., 2020; Li et al., 2021; Zhao et al., 2021). This might be indicative of oxidative injury among animals from gold mining areas in Anka LG.

Increased in LDH reported in this study is also similar to a recent report on Rabbit (Mumtaz et al., 2019). This likely shows that chickens in gold mining areas are under heat stress due to perturbation in blood metabolites with resultant increased in LDH. This is in line with a recent study where induced oxidative stress increase LDH activities in broilers (Chen et al., 2021).

TAC, a more reliable biomarker than each element alone was evaluated which shows that TAC was significantly reduced in chicken from gold mining area. This is in congruent with a recent report that induced oxidative stress in poultry leading to a significant reduction in the TAC (Surai et al., 2021). It also in line with other studies on fish where mercury exposure led to the elevated lipid peroxidation with a concurrent reduction of TAC in the plasma (Kuras et al., 2019). A recent related study in mice shows that concomitant elevated amounts of inorganic mercury resulted in a substantial reduction in the cortex's TAC (Teixeira et al., 2018). Consequently, reduced antioxidants defences and overexpression of free radicals reported among chicken from gold min-

ing sites might create an imbalance which work in synergy to destroy important macromolecules (DNA, proteins and lipids). This is an indication that potential mercury exposure in gold mining areas might be detrimental to physiological status of chickens.

Heat shock proteins (HSPs) were the first molecular chaperones discovered, and they help fold newly synthesized proteins, reverse and inhibit aggregation of non-native and misfolded proteins. This helped in the disintegration of terminally misfolded proteins, while sustaining protein homeostasis and cellular proteostasis under stress and normal physiological conditions (Klaips et al., 2018; Murugesan et al., 2017; Shehata et al., 2020).

However, in stressful conditions, when the risk of proteins and other macromolecules malfunctioning is increased, the necessity for molecular chaperones becomes even more critical. Heat shock proteins, particularly HSP70, have been recommended as good stress biomarkers and has been used in several ecotoxicological studies in the last two decades (de Souza et al., 2020; Greene et al., 2019; Morales et al., 2011; Morris et al., 2013; Murugesan et al., 2017; Tkáčová and Angelovičová, 2012; Yang et al., 2020; Yang et al., 2020). The HSP70 could be used to monitor stress levels in many avian species because it is widely expressed in internal organs, blood, and feathers, and its synthesis is triggered by a variety of other stressors such as water deprivation, infection, toxins, stress and exposure to chemicals like mercury (Greene et al., 2019).

The HSP 70 levels that were found to be significantly higher in poultry from gold mining sites in this study could be linked to increased mercury levels in their serum. This is similar to previous results on methylmercury and mercury exposure in the *Sparus Aurata* Fibroblast-1 (SAF-1) cell line and erythrocytes (derived from the fin of sea bream) and Yellow catfish (*Pelteobagrus fulvidraco*) that resulted in a considerable increase in HSP 70 expression (Morcillo et al., 2016a; Morcillo et al., 2016b; Sun et al., 2018). The HSP70 mRNA and protein were both upregulated in broiler feathers, gut, and blood in response to intracellular and systemic stress in this study, which is similar to other studies where HSP70 mRNA and protein were both upregulated in broiler feathers, gut, and blood in response to intracellular and systemic stress (Greene et al., 2019). Heat stress has also been linked to increased expression of HSP 70 in the liver and hypothalamus of chickens, according to other studies (Flees et al., 2017; Rajaei-Sharifabadi et al., 2017; Rajaei-Sharifabadi et al., 2017). Our result is in line with a recent study where exposure to mercury chloride in male Hyline brown chicken activated HSP 70 protein expression (Fan et al., 2020). Other investigations have found a higher level of HSP 70 in the midgut of *Rhinocricus padbergi* (Diplopoda), chick embryos, after exposure to metallic pesticides, which is consistent with our findings (de Souza et al., 2020; Papaconstantinou et al., 2003). Another study found that short-term acute exposure to environmental stressors like endocrine disrupting pollutants which include variation in temperature, 4-nonylphenol, butyl benzyl phthalate (BBP), diethylhexyl phthalate, cadmium chloride, bisphenol A, and ethinylestradiol significantly increased HSP 70 mRNA in *Chironomus riparius* (Morales et al., 2011).

As a result of our findings, subsequent research have found that HSP production increases in response to stress as a biological strategy to protect various proteins from harm. As a result, during times of stress, increased HSP expressions were found in practically all of the chicken's major organs (Murugesan et al., 2017). Stresses like hyperthermia, oxidative damage, or physical/chemical injury can substantially and quickly upregulate HSP expression (De Maio, 1999). Finally, studies reveal that HSP 70 may play a cytoprotective role during stress by suppressing the aggregation of non-native and misfolded proteins via a mitochondrial mechanism (Klaips et al., 2018; Murugesan et al., 2017; Shehata et al., 2020; Xu et al., 2017).

Mercury is regarded as immunotoxicant since several studies have reported that one of the essential mechanism of mercury toxicity is the inflammatory response (Ahn et al., 2018; Bjørklund et al., 2020; Fan et al., 2020; Han et al., 2021; Pollard et al., 2019; Renu et al.,

2021; Sun et al., 2018; Zhao et al., 2021). It is worth noting that specific signalling pathways, such as the NF κ B pathway play a significant part in the anti-inflammatory effects of antioxidants (Almeer et al., 2019; Fan et al., 2020; Iwai-Shimada et al., 2016; Jiang et al., 2018). As an important transcription factor, NF κ B serves as a paramount link between inflammation and oxidative stress (Blaser et al., 2016; Fan et al., 2020). Following oxidative stress, NF κ B increases the production of inducible nitric oxide synthase (iNOS) and the subsequent synthesis of Nitric oxide (NO), which then activates the NF κ B signalling pathway, resulting in the release and expression of particular inflammatory factors. HgCl₂ causes inflammation by boosting the release of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α via the NF κ B signalling pathway, according to studies (Ahn et al., 2018; Blaser et al., 2016; Fan et al., 2020; Renu et al., 2021).

Activation of cytoplasmic multiprotein complex called inflammasome by a repertoire of endogenous and exogenous danger signals triggered the maturation and secretion of interleukin (IL)-1 β as well as apoptotic and pyroptotic cell death (Ahn et al., 2018; Kim et al., 2020; Zheng et al., 2020). This process usually rely on NF κ B signaling to induce the transcription of the inflammasome components. Dysregulated inflammasome activation is detrimental to the host response to tissue damage and may contribute to compromised health in animals (Kim et al., 2020; Zheng et al., 2020).

Tumour necrosis factor (TNF) is a pro-inflammatory cytokine that is crucial for cellular homeostasis and immunity; its dysregulation generally results in inflammation (Blaser et al., 2016). It is impossible to overstate the importance of TNF as the main modulator in regulating apoptosis, necrosis and cell survival in many cell types and tissues. TNF has a direct impact on the regulation of nuclear factor kappa light chain enhancer of activated B cells (NF κ B) and JUN N-terminal kinase (JNK), which has led to a better understanding of TNF's growing role as a regulator of both reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS influences signalling pathways that lead to NF κ B activation and antioxidant protein overexpression, suggesting that TNF and ROS influence each other in a positive feedback loop (Blaser et al., 2016).

ROS play key role in cell death and survival triggered by TNF- α signaling; the primary source in ROS generation necessary for cell death induced by TNF- α is the mitochondria. This is because ROS modulator 1 (Romo1) was involved in TNF- α -induced ROS production and it is found in the outer membrane of the mitochondria to sense the signal produced by the TNF- α which consequently induces ROS generation (Kim et al., 2010; Yang et al., 2020).

Earlier reports have opined that cell death, caspase activation as well as ultrastructural perturbations in various tissues have been reported as a result of exposure to heavy metals in various forms. Inflammation involving TNF- α and pro-inflammatory cytokines like IL-1 β and IL-6 have been reported to be important in heavy metal toxicity (Chen et al., 2022; Fan et al., 2020; Renu et al., 2021). Consequently, some heavy metals have been reported to activate the TNF- α / NF κ B pathway and induced inflammatory responses via increasing the expression of Haem oxygenase 1 (HO-1), IL-1 β , inducible nitric oxide synthase (iNOS), Cyclooxygenase-2 (COX2) (Campbell et al., 2021; Chen et al., 2022; Fan et al., 2020; Renu et al., 2021). A recent study also suggested that Hg²⁺ exposure can cause immunomodulatory response in birds by dysregulating the immune cell amplification and cytokine proliferation, this might finally lead to disorder and perturbation of the avian immune system (Han et al., 2021). Another study also reported that inorganic mercury induced spleen damage is triggered by autophagic cell death through phosphatidylinositol 3-kinase (PI3K)-AKT pathway (Zhao et al., 2021). These have demonstrated that mercury from gold mining areas may constitute a major hazard to health and the environment in general.

In this study, TNF- α , IL-1 β and IL-6 levels are significantly higher in the serum of mercury-exposed chicken from mining areas when compared with those assessed in the control group. This result is in congruent with a recent study that HgCl₂ exposure in Hy-Line brown chicken lead to overexpression of pro-inflammatory cytokines which include

interleukin (IL-1 β , IL-6 and TNF- α) (Fan et al., 2020). It is also similar to a study involving yellow catfish (*Pelteobagrus fulvidraco*) where Hg²⁺ exposure upregulated IL-1 β and TNF- α mRNA levels in the spleen (Sun et al., 2018) and also in agreement with earlier reports in zebrafish larvae (Zhang et al., 2016), suggesting that Hg²⁺ might trigger an inflammatory response (Björklund et al., 2020). Other animal studies suggested that exposure to mercury markedly elevated the expression of numerous pro-inflammatory mediators, pro-apoptotic genes (IL-1 β , IL-6 and TNF- α), innate and adaptive immune cells are increased (Cauvi et al., 2017; Pollard et al., 2019; Zhao et al., 2020). Thus, we opined as other studies that mercury exposure in chicken through any means might disrupt inflammatory responses, that will likely cause fatal health effect (Ahn et al., 2018; Fan et al., 2020; Pollard et al., 2019; Zhao et al., 2020).

Subsequently increased proliferation of pro-inflammatory cytokines like IL-1 β , IL-6 and TNF- α reported from chickens in gold mining areas might be mercury induced through the activation of NF κ B signalling pathway. We suggest that these pro-inflammatory cytokines might have an important role in the selective cellular damage caused by methylmercury. Additionally, results from this study did indicated that the elevated TNF- α activity triggered by methylmercury might culminate into cell death, as TNF- α interact with its receptor after being released extracellularly (Iwai-Shimada et al., 2016). Finally, this study corroborated a recent report which revealed that exposure to mercury might lead to disorders of the avian immune system which might cause unexpected side effects as it might disrupt bird immune cell generation and cytokine synthesis (Ahn et al., 2018; Han et al., 2021).

Conclusions

Environmental Hg contamination continue to be a global issue with rising risk to wildlife and human populations in many regions of the world because of human population expansion industrialisation, and artisanal and small-scale gold mining (ASGM). Mercury exposure because of ASGM affect most farmed animals in food chains. Result from this study showed elevated levels of mercury in free ranging chicken (*Gallus Gallus domesticus*) from gold mining areas in Zamfara State Nigeria. This might be due to the presence of Hg in the environment due to ASGM activities in those areas. Elevated mercury in these free-ranging chicken might be due to mercury bioaccumulation from local food stuff. This study also showed that potential exposure to mercury through gold mining activities in Zamfara have the potential health effect as they induce immune response, heat shock and oxidative stress in free ranging chicken in Anka LG. Finally, we opined that chicken meat products might serve as an important mercury exposure source for the people of Anka LG.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRedit authorship contribution statement

Zainab Abdulmalik: Data curation, Writing – original draft, Writing – review & editing. **Muftau Shittu:** Conceptualization, Methodology, Software, Visualization, Investigation, Supervision, Software, Validation, Writing – review & editing. **Sani Adamu:** Conceptualization, Methodology, Software, Visualization, Investigation, Supervision, Software, Validation, Writing – review & editing. **Suleiman Folorunsho**

Ambali: Conceptualization, Methodology, Software, Software, Validation, Writing – review & editing. **Bolaji Fatai Oyeyemi:** Data curation, Writing – original draft, Writing – review & editing.

Data Availability

Data will be made available on request.

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