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ORIGINAL ARTICLE

TOXIC EFFECTS OF AFLATOXIN B1 ON SOME SERUM HORMONES LEVELS IN TYPE 2 DIABETES FEMALE PATIENTS

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Abstract

Background: Aflatoxin B1 (AFB1) is a potent mycotoxin known to exert toxic effects on various biological systems, including the endocrine system. Women with diabetes mellitus type 2 (DMT2) may be particularly vulnerable to hormonal disturbances, especially when exposed to environmental toxins like AFB1. The aim of this present study was to investigate an examination of AFB1 in patients' blood with DMT2 and the correlation between some specific Hormone levels of patients.

Materials & Methods: A case-control study was worked in Diabetes and Endocrinology Center at Imam Al-Hussein Hospital, from September to December 2024. A total of 100 women (50 DMT2 patients and 50 healthy controls) aged 35–65 years were enrolled. Blood samples were collected to measure serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, Estradiol (E2), and testosterone using the Abbott Architect i2000SR system. AFB1 levels were detected using Thin Layer Chromatography (TLC) and quantified by HPLC. In order to perform the statistical analysis, version 26 of SPSS was utilized.

Results: AFB1 demonstrated a positive correlation with DMT2 (r = 0.319), and E2 levels showed a significant decrease, while testosterone and prolactin levels exhibited a notable increase in the toxin-exposed group, additionally, both LH and FSH levels were significantly reduced in patients exposed to the toxin. Assessment of receiver Characteristics curve (ROC) analysis showed that AFB1 Toxin is considered reasonably excellent for DMT2 patients with a sensitivity 90.000% and a specificity of 97.500%.

Conclusion: Exposure to AFB1 may contribute to hormonal dysregulation in women with DMT2, suggesting a potential link between environmental toxins and endocrine complications in diabetic patients.

KEY WORDS: Aflatoxin B1; Diabetes Mellitus; Endocrine hormones; Estradiol; Prolactin; Testosterone; Type 2 diabetes mellitus

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INTRODUCTION

Aflatoxins are a group of highly toxic and carcinogenic secondary metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*, fungi that commonly contaminate a wide range of agricultural products, especially grains, nuts, and spices .[1] Among these, Aflatoxin B1 (AFB1) is the most potent and widely studied due to its strong hepatotoxic, mutagenic, and immunosuppressive effects, the chronic exposure to AFB1, even at low levels, has been linked to various health conditions, including liver cancer, immune dysfunction, and hormonal imbalances .[2] In recent years, there has been increasing concern regarding the endocrine-disrupting potential of AFB1, particularly its influence on reproductive and metabolic hormones. AFB1 may interfere with hormonal synthesis, secretion, and regulation by acting on the hypothalamic-pituitary-gonadal (HPG) axis. This disruption can result in significant alterations in the levels of hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, testosterone, and estrogen, which play critical roles in female reproductive health and overall hormonal balance.[3]

On the other hand, DMT2 is a long-term metabolic condition have feature insulin resistance, impaired glucose metabolism, and endocrine dysfunction.

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Women with DMT2 are particularly vulnerable to hormonal dysregulation due to a combination of metabolic stress, inflammation, and possible exposure to environmental toxins like AFB1. However, there is limited literature exploring the combined effect of AFB1 exposure and DMT2 on hormonal profiles, especially among women .[4] Given these findings, this study aimed to investigate the toxic effects of Aflatoxin B1 on the levels of specific hormones (LH, FSH, prolactin, testosterone, and E2) in women suffering from DMT2 blood serum. Understanding this relationship is crucial for assessing the health risks associated with AFB1 exposure in diabetic populations.

MATERIALS AND METHODS

This study was a case-control investigation conducted at the Diabetes and Endocrinology Center at Imam Al-Hussein Hospital. The data was collected and recorded from 1st September 2024 to 30th December 2024. A total of 100 women aged between 35 and 65 years were enrolled in the study. The case group included 50 women diagnosed with DMT2 based on the American Diabetes Association (ADA) criteria, while the control group consisted of 50 apparently healthy women matched for age with no known history of diabetes or hormonal disorders.

Sterile vacutainer tubes were used to collect 10 milliliters of venous blood from each participant following informed consent. Once the blood had clotted, the serum was separated by centrifugation at 3000 rpm for 10 minutes. Until analysis, serum samples were kept at -20°C. Chemiluminescent immunoassay methods were utilized on the Abbott Architect i2000SR system to measure a hormonal parameter including LH, FSH, Prolactin, E2, and Testosterone. According the detection and confirmation of AFB1 presence in serum were initially carried out using TLC, employing standard AFB1 as a reference under UV light (365 nm), For determination The concentration of AFB1 in serum was using HPLC with fluorescence detection ,the method was validated according to internationally recognized analytical standards.

Data analysis was performed using **SPSS version 26**. Results were expressed as mean ± standard deviation (SD). An independent t-test was used to compare variables between diabetic and control groups, while Pearson correlation was used to assess relationships between AFB1 levels and hormonal markers. A p-value < 0.05 was considered statistically significant. [5] The ethical study protocol was approved by the Research Committee of the College of Applied Medical Sciences, university Of Karbala. All patients involved in this work were informed and prior to sample collection, each participant verbally agreed. Data were analyzed using IBM SPSS Statistics (version 23). Statistical analyses included descriptive statistics, mean calculations, and standard deviations. Data normality was verified using the Shapiro-Wilk test, and homogeneity of variance using the Levene test. To test for differences between groups, the independent t-test and the Mann-Whitney test were used, and for multiple comparisons, the ANOVA with Scheffe and Duncan tests were used. Relationships were analyzed using the Pearson and chi-square tests. ROC analyses were performed to determine parameter cutoff values using the Youden index, and a GraphPad Prism 9 was employed to construct the graphs. A P value of less than 0.05 was regarded as statistically significant.

RESULTS

A total of 100 women (50 DMT2 patients and 50 healthy controls) aged 35–65 years were enrolled. The results revealed a significant reduction in the E2 levels of serum groups. Patient with toxin (30.978 ng/dl) and patient without toxin (66.000 ng/dl) , patients exposed to aflatoxin had lower levels of E2 than the control without toxin group (55.690 ng/dl) indicating a cumulative effect between the disease and the toxin. There is also a significant difference (0.00143) as shown in the Table 1.

Table 1: The Comparisons between Research Groups According to the E2 Parameter.

Groups	Mean ng/dl	Std. Deviation	P-Value
Patient with Toxin	30.978	23.129	0.00143*
Patient without Toxin	66.000	36.765	
Control with Toxin	70.871	31.836	
Control without Toxin	55.690	37.878	

**=Significant p<0.005; The significant variation between the study groups.*

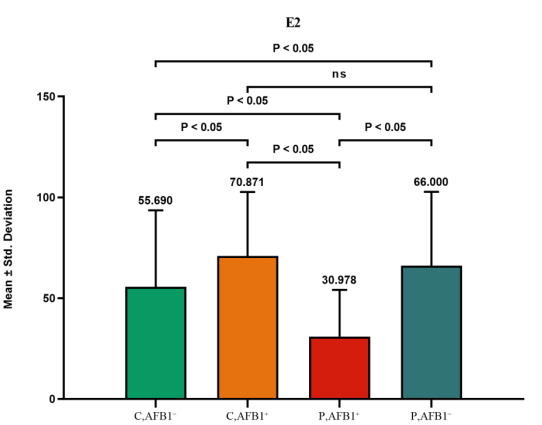

Figure 1: The Comparisons among Research Groups According to the E2 Parameter.

The results showed significant differences in LH hormone levels between the studied groups. The group patient with toxin recorded a significant decrease in mean levels (12.884 ng/dl) compared to the group of patient without toxin (13.106 ng/dl), with a significant difference (*P*-Value = 0.01452), indicating that the toxin may have an inhibitory effect on hormone secretion in patients ,In contrast, the group of control with toxin recorded the lowest levels (6.475) compared to all other groups, including control without toxin (13.054 ng/dl), confirming the clear inhibitory effect of the toxin on LH secretion in the absence of disease. as shown in the Table 2.

Table 2: The Comparisons between Research Groups According to the LH Parameter.

Groups	Mean ng/dl	Std. Deviation	*P*-Value
Patient with Toxin	12.884	9.012	0.01452*
Patient without Toxin	13.106	9.731	
Control with Toxin	6.475	2.251	
Control without Toxin	13.054	7.744	

**= significant p< 0.005; The significant variation between the study groups.*

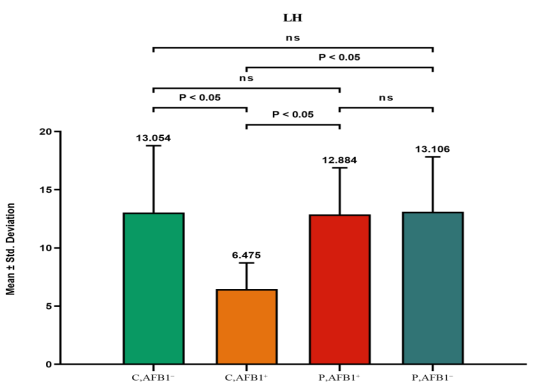

Figure 2: The Comparisons among Research Groups According to the LH Parameter.

The results showed significant differences in FSH levels between the studied groups. The group of Patient with Toxin recorded a significant decrease in mean levels (35.703 ng/dl) compared to the group of patient without toxin (46.272 ng/dl), with a significant difference (*P*-Value = 0.00386), indicating that the toxin may have an inhibitory effect on hormone secretion in patients ,In contrast, the group of control with toxin recorded the lowest levels (4.216 ng/dl) compared to all other groups, including control without toxin (35.264 ng/dl), confirming the clear inhibitory effect of the toxin on FSH secretion in the absence of disease, . as shown in the Table 3.

Table 3: The Comparisons between Research Groups According to the FSH Parameter.

Groups	Mean (ng/dl)	Std. Deviation	*P*-Value
Patient with Toxin	35.703	28.884	0.00386*
Patient without Toxin	46.272	40.085	
Control with Toxin	4.216	2.584	
Control without Toxin	36.264	39.103	

**= significant p< 0.005; The significant variation between the study groups.*

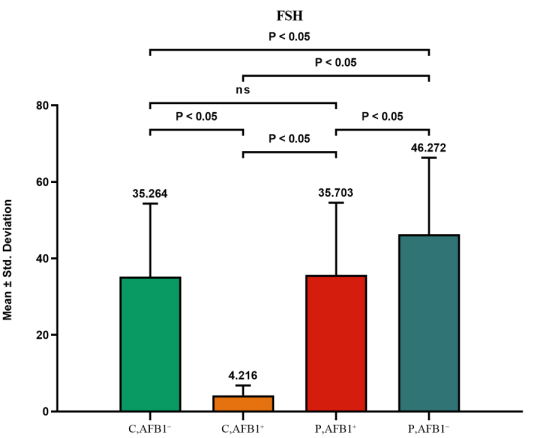

Figure 3: The Comparisons among Research Groups According to the FSH Parameter.

The results showed significant differences in prolactin levels among the studied groups. The group of patients with toxin recorded a higher mean value (10.849 ng/dl) compared to the group of patients without toxin (8.316 ng/dl), with a s significant difference (*P*-Value = 0.04495). This suggests that exposure to AFB1 may be associated with increased prolactin levels in diabetic patients ,on the other hand, both control with toxin (10.182 ng/dl) and control without toxin (10.461 ng/dl) groups showed no significant difference, as they shared the same group letter (a) in the Duncan test. This indicates that the effect of the toxin on prolactin secretion was more evident in patients than in healthy individuals as shown in the Table 4.

Table 4: The Comparisons between Research Groups According to the Prolactin Parameter

Groups	Mean (ng/dl)	Std. Deviation	Duncan Test	*P*-Value
Patient with Toxin	10.849	4.887	A	0.04495*
Patient without Toxin	8.316	4.520	B	
Control with Toxin	10.182	4.785	A	
Control without Toxin	10.461	4.560	A	

**= significant p< 0.005; The significant variation between the study groups.*

The results showed significant differences in testosterone levels between the studied groups (*P*-Value = 0.04742). The group of patients with toxin recorded the highest mean testosterone level (31.978 ng/dl), followed by the group of control with toxin (30.786 ng/dl). In contrast, the group of patient without toxin (26.172 ng/dl) and the group of control without toxin (2 9.676 ng/dl) showed significantly lower levels , Table 5.

Table 5: The Comparisons between Research Groups According to the Testosterone Parameter

Groups	Mean (ng/dl)	Std. Deviation	Duncan test	*P*-Value
Patient with Toxin	31.978	13.718	A	0.04742*
Patient without Toxin	26.172	9.913	B	
Control with Toxin	30.786	7.140	A	
Control without Toxin	29.676	12.034	B	

**= significant p< 0.005; The letters' differences suggest that there is significant variation between the study groups.*

The overall Area Under Curve (AUC) of E2 , FSH and AFB1 was 70.550% , 71.525% , 96.725% respectively , "These parameters are considered reliable indicators used to predict the increased risk of developing DMT2, making them an important tool for early detection and risk management.", while the sensitivity and specificity of E2 were 68.000% and 82.500% respectively. Also, the sensitivity and specificity of FSH were 76.000% and 70.000% respectively, and the sensitivity and specificity of AFB1 were 92.000% and 97.500% respectively as in Table 6.

These statistically significant markers can be used to diagnose positive cases of the condition according to the ROC curves generated by graphing the sensitivity against specificity.

Estradiol hormone is considered an Average accuracy indicator for Predicting the likelihood of developing DMT2, with a sensitivity of 68.000% and specificity of 82.500% at the chosen Cut-Off, this statistically significant marker can be used to diagnose positive cases of condition (Figure 4)

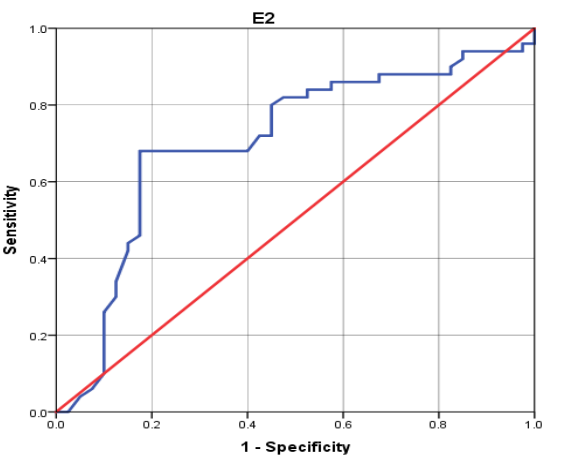

Figure 4: Model prediction of subject working characteristic curves according to the E2 parameter.

With a sensitivity of 76.000% and a specificity of 70.000% at the chosen Cut-Off, FSH hormone is considered a good indicator for predicting the likelihood

Table 6: Model prediction of subject working characteristic curves according to the research parameters.

Metrics		AFB1	E2	FSH
Std. Error		0.019	0.058	0.059
Asymptotic Sig.		0.003	0.001	0.004
Asymptotic 95% Confidence Interval	Lower Bound	0.929	0.592	0.600
	Upper Bound	1.000	0.819	0.831
Cutoff Point		6.250	26.000	7.495
Area Under Curve (AUC)		96.725%	70.550%	71.525%
Sensitivity		92.000%	68.000%	76.000%
Specificity		97.500%	82.500%	70.000%

of developing DMT2. This statistically significant marker can be used to diagnose positive cases of the condition (Figure 5).

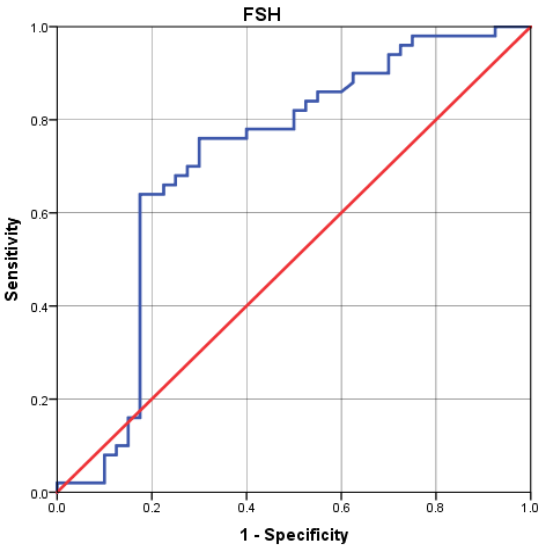

Figure 5: Model prediction of subject working characteristic curves according to the FSH parameter

Aflatoxin B1 toxin is considered a reasonably excellent indicator for DMT2 patients with a sensitivity of 95.83% and specificity of 95.45% at the chosen Cut-Off, this statistically significant marker can be used to diagnose positive cases of condition (Figure 6)

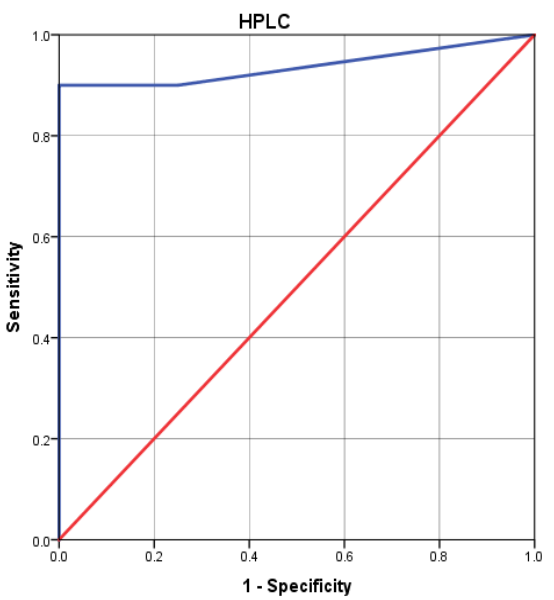

Figure 6: Model prediction of subject working characteristic curves according to the HPLC (AFB1) parameter

DISCUSSION

AFB1 demonstrated a positive correlation with DMT2 (r = 0.319), and E2 levels showed a significant decrease, while testosterone and prolactin levels exhibited a notable increase in the toxin-exposed group, additionally, both LH and FSH levels were significantly reduced in patients exposed to the toxin. Assessment of receiver Characteristics curve (ROC) analysis showed that AFB1 Toxin is considered reasonably excellent for DMT2 patients with a sensitivity 90.000% and a specificity of 97.500%. In one study the results demonstrated a significant decrease in serum E2 levels in goats exposed to AFB1 during both the peri-ovulatory and luteal phases.[6] The significant reduction in serum E2 levels demonstrated that exposure to AFB1 leads to a dose-dependent suppression of estradiol-17ß secretion during both the luteal phase and synchronized estrus. This decrease is likely due to the direct toxic effect of AFB1 on granulosa cells within the ovarian follicles ,Granulosa cells play a critical role in E2 biosynthesis .

Also, the findings were the same as those of another study of [7], in which it is reported a significant reduction in serum E2 levels in reproductively mature pigs exposed to varying dietary concentrations of AFB1. In this context, the reduction in estradiol levels may be attributed to the toxic effects of AFB1 on the testes, particularly on the Leydig and Sertoli cells, which play crucial roles in steroidogenesis , E2 is also synthesized in the male testis via aromatization of testosterone by the enzyme aromatase (CYP19A1). AFB1 exposure may inhibit this enzymatic activity either by direct cytotoxicity or through suppression of gene expression, thereby reducing the conversion of testosterone to E2, also AFB1 exposure may inhibit this enzymatic activity either by direct cytotoxicity or through suppression of gene expression, thereby reducing the conversion of testosterone to E2, the observed decline in E2 levels in male pigs exposed to AFB1 underscores the endocrine-disrupting potential of this mycotoxin. This finding aligns with existing literature suggesting that AFB1 not only affects reproductive hormone levels in females but also poses significant risks to male reproductive health through inhibition of steroid hormone biosynthesis.

On the other hand, a study published in the journal BMC Endocrine Disorders indicated an inverse relationship between E2 levels and the risk of developing DMT2. Low blood E2 levels were found to be associated with increased insulin resistance and impaired glucose metabolism, contributing to the development of DMT2. This effect is attributed to the physiological role E2 plays in regulating glucose homeostasis by enhancing cell sensitivity to insulin, reducing inflammation, and improving glucose uptake in peripheral tissues[8], if AFB1 inhibits E2, this inhibition could in turn contribute to an increased risk of developing DMT2, or worsening the condition in patients with it.

The result of this study agreed with a study conducted on dairy buffaloes, exposure to AFB1 resulted in

a significant decrease in LH levels.[9] The researchers attributed this effect to the oxidative stress caused by the toxin, which disrupts the function of the hypothalamic-pituitary-gonadal (HPG) axis. Liver dysfunction resulting from toxin accumulation may also impair hormone metabolism and affect feedback mechanisms, exacerbating hormonal imbalance and impairing reproductive performance. Also ,This study agreed with who showed that early exposure to AFB1 in female rats results in a marked decrease in LH levels.[10] This decrease was explained by disturbances in the hypothalamic-pituitary-gonadal axis, resulting from oxidative stress and epigenetic changes. These results reinforce the hypothesis that AFB1 exerts an inhibitory effect on reproductive hormonal regulation even in the early stages of development.

Also another study that agreed[6] the study conducted on goats indicated that exposure to AFB1 during critical stages of the reproductive cycle, such as the luteal phase and ovulation, led to disturbances in reproductive hormones, particularly estradiol and progesterone, although the study did not directly measure LH, the observed hormonal changes reflect a dysregulation of the hypothalamic-pituitary-gonadal axis, supporting the current study's findings that AFB1 contributes to LH suppression and negatively impacts fertility. On the other hand, the study revealed a close relationship between DMT2 and abnormal reproductive hormone levels, particularly LH. An Iraqi study demonstrated that DMT2 patients suffer from significantly lower LH concentrations compared to healthy individuals, this is attributed to a disruption in the hypothalamic-pituitary-gonadal axis (HPG axis) resulting from insulin resistance and chronic oxidative stress associated with the disease.[11]

When the two effects are combined, an overlap in pathological mechanisms is observed, as both diabetes and aflatoxin B1 contribute to impaired neuronal and hormonal regulation of the HPG axis. Both also enhance oxidative stress and chronic inflammatory responses, leading to further inhibition of LH secretion and potentially exacerbating fertility disorders, especially in diabetic women exposed to aflatoxin.

The results of our Study are consistent with an other study[12], as a significant decrease in FSH concentration was observed in females exposed to AFB1. This decrease was explained by a dysregulation of the hypothalamic-pituitary-ovarian axis (HPG axis), which causes inhibition of FSH secretion from the pituitary gland. This effect may result from a negative feedback response resulting from the impact on ovarian follicle growth, which could directly affect fertility and reproductive function. Also another study [13] ,also supported our hypothesis by examining women suffering from infertility. The researchers noted that these women were more susceptible to exposure to aflatoxin through food, and this was associated with a decrease in FSH levels, indicating an indirect relationship between AFB1 and this hormone disorder in women.

One study reported that oral exposure to AFB1 in adult male rats resulted in a significant increase in serum prolactin levels ($P < 0.001$), and the current study's findings are consistent with these findings.[13] The toxin's disruptive effects on the hypothalamic–pituitary axis, which may disrupt the normal regulation of prolactin secretion, were the cause of this rise, according to the authors. This is consistent with the fact that diabetic female patients exposed to AFB1 had higher levels of prolactin. This suggests that the toxin may stimulate prolactin secretion regardless of gender, possibly through shared neuroendocrine mechanisms. In some other study it is shown, that increased testosterone levels in diabetic females exposed to aflatoxin, the study by reported that AFB1 impaired ovarian function and possibly reduced sex hormone levels. This difference may be due to species, exposure type, or the metabolic condition (e.g., diabetes), but both studies confirm AFB1 disruptive impact on hormonal balance.[14]

CONCLUSION

Exposure to AFB1 may contribute to hormonal dysregulation in women with DMT2, suggesting a potential link between environmental toxins and endocrine complications in diabetic patients.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	ZAM, SAA
Acquisition, Analysis or Interpretation of Data:	ZAM, SAA
Manuscript Writing & Approval:	ZAM, SAA

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.