DISTURBED SERUM SPHINGOSINE-1-PHOSPHATE LEVELS COULD PREDICT DISTURBED LIVER AND KIDNEY FUNCTIONS IN APPARENTLY HEALTHY PERSONS CHRONICALLY EXPOSED TO FUMONISINS

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ABSTRACT

Objectives: The current study tried to determine the effects of exposure to fumonisins on kidney and liver functions tests and sphingolipid metabolites.

Patients & Methods: 80 apparently healthy participants of Area 1(A1) that was considered as area heavily contaminated by fumonisins and Area 2 (A2) that was considered as low contamination area. Ten urban volunteers were enrolled as a control group. All participants underwent full history taking, complete clinical examination with special regard to liver and kidney and gave blood samples for estimation of serum levels of aspartate transaminase (AST), alanine transaminase (ALT), urea, creatinine, and plasma lipid profile, and ELISA estimation of plasma sphingosine-1-phosphate (S1P) levels

Results: The mean levels of serum AST, ALT, urea, and creatinine were significantly higher, while plasma levels of high-density lipoprotein (HDL-c) and S1P were significantly lower in samples of study participants in comparison to samples of controls, with significant differences between participants of both groups. Duration of exposure showed a positive correlation with estimated serum AST, ALT, urea, and creatinine while showing a negative correlation with estimated levels of plasma HDL-c and S1P. Estimated plasma S1P showed a negative correlation with participants' age and serum levels of AST, ALT, urea, and creatinine while showing a positive significant correlation with plasma levels of HDL-c. Regression analysis defined long duration and severity of exposure and older age as predictors for low plasma S1P, which is a predictor for impaired liver enzymes levels, kidney affection, and low plasma HDL-c.

Conclusion: Chronic fumonisin exposure deleteriously, but gradually affects kidney and liver functions, either directly or through disturbing the sphingolipid metabolism and depletion of sphingosine. Low serum levels of S1P were found to correlate with renal and hepatic affection and could be used as an early marker for the detection of such changes.

INTRODUCTION

Fumonisins that were discovered in 1988 are a group of naturally occurring toxins produced by *fusarium* pathogenic fungi (Wan et al., 2013). The fumonisins are the common worldwide mycotoxins that mainly contaminate maize (Müller et al., 2017). Fumonisin B_1 is the most widely distributed and the most toxic for being is water soluble and stable at room temperature and to light (Yamazoe et al., 2017).

When maize contaminated by fumonisins was consumed fumonisins cause a wide spectrum of diseases that involve liver and kidney toxicity in many species, carcinogenicity for rodents, vasculature, and brain in equine leukoencephalomalacia, and lung in porcine pulmonary edema syndrome (Riley et al., 2018). Health concerns in humans are multiple, where maternal consumption of fumonisin-contaminated maize during early pregnancy was found to be associated with an increased risk for fetal neural tube defects (Waes et al., 2009). Also, fumonisins had been associated with the development of esophageal cancer (Lombard et al., 2014). Moreover, infants and young children are the most vulnerable to the effects of fumonisins, which affect children's health and growth, especially those who were weaned onto maize-based foods (Chen et al., 2018).

The de novo sphingoid base biosynthesis occurs in the endoplasmic reticulum, mitochondria, and its associated membranes, where ceramide synthases (sphinganine/ sphingosine N-acyltransferase) incorporate serine and a palmitoyl-CoA into 3-ketosphinganine followed by *N*-acylation to form dihydroxy ceramide which in turn is desaturated to ceramide to be incorporated in the biosynthesis of sphingolipids and glycosphingolipid (Merrill, 2011) which arrive at their destinations via vesicular transport, transport proteins, and sorting mechanisms at the *trans*-Golgi network (Hernández-Corbacho et al., 2017). Turnover of sphingolipids occurs by lysosomal hydrolases, autophagosomes, and other organelles to release the sphingoid base, mainly sphingosine that is salvaged via Ceramide synthase or phosphorylated by sphingosine kinases to 1-phosphates to function in cell signaling or efflux from the cell (Harrison et al., 2018).

Fumonisin B1 shared chemical similarity with sphingoid bases (Fig. 1) and thus gains its toxicity through inhibition of ceramide synthase causing an accumulation of sphingoid bases (Merrill et al., 1993), inhibition of protein phosphatases (Fukuda et al., 1996), and interaction with arginosuccinate synthase, a urea cycle enzyme (Jenkins et al., 2000).

Fumonisin exposure is the highest in rural populations that consume large amounts of maize and maize products (Chen et al., 2018). Thus, the current study aimed to determine the effects of community pollution by fumonisins in two distinct rural areas diffident in the severity of contamination as judged by the extent of dependence on maize on kidney and liver functions tests and sphingolipid metabolites.

Design:

Prospective comparative study

Setting:

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PATIENTS AND METHODS

According to the agricultural map of the Qalyubia government two rural areas were chosen for case collection; Area 1(A1) has wide areas that had been planted by the maze and so was considered as area heavily contaminated by fumonisins, either by direct exposure during fieldwork, home-storage of the maze or using its leaflets as a ration for animals or indirectly through air pollution by fumonisins. Area 2 (A2) has minimal areas that had been planted with a maze and so was considered as a low contamination area.

Exclusion criteria

Age younger than 18 and older than 60 years, presence of liver or kidney impairment secondary to any pathology, presence of chronic anemia or its predisposing factors as polymenorrhea in females, hookworm infestations, bleeding urinary or gastrointestinal diseases, refusal to participate in the study.

Inclusion criteria

Apparently healthy persons working in maze-related agriculture or other duties and were free of exclusion criteria.

Grouping

1. Group A1: included 40 persons fulfilling the inclusion criteria and receding in Area 1

2. Group A2: included 40 persons fulfilling the inclusion criteria and receding in Area 2

3. Group C: included 10 persons free of exclusion criteria, receding in an urban area, and had no relation to maze planting or manipulation as a control group

Study protocol

The study participants were collected during one of the arranged health provision visits arranged by Benha University hospital for inhabitants of rural areas. All participants underwent full history taking, complete clinical examination with special regard to liver and kidney.

Blood Sampling

All participants gave random blood samples under complete aseptic condition and divided into the following parts:

1. The first part was put in a tube containing sodium fluoride (2 mg sodium fluoride/ ml blood) to prevent glycolysis for estimation of blood glucose levels.

2. The second part was divided into two parts and both were collected in lithium-heparin-containing tubes. One part was used for estimation of plasma lipid profile and the 2nd part was stored at $-20^{o}C$ for estimation of plasma sphingosine-1-phosphate (S1P) levels.

3. The third part was collected in a plain tube, allowed to clot, centrifuged at $1500 \times g$ for 15 min, and the se-rum samples were collected for estimation of liver enzymes, urea, and creatinine.

Estimated parameters

1. Blood glucose levels were estimated by the glucose oxidase method (Tinder, 1969) using the BT1500 Automatic biochemistry analyzer (SPAN Diagnostics, Gujarat India).

2. Serum liver enzymes; aspartate transaminase (AST) and alanine transaminase (ALT) were estimated by photoluminescence method (Reitman

& Frankel, 1957) using BT1500 Automatic biochemistry analyzer (SPAN Diagnostics, Gujarat India).

3. Serum urea was estimated by Urease-modified Berthelot reaction (Fawcett & Scott, 1960) and serum creatinine was estimated by measuring the rate of formation of a creatinine-picrate colored complex in a fixed time (Taussky, 1961) using BT1500 Automatic biochemistry analyzer (SPAN Diagnostics, Gujarat India).

4. Plasma total cholesterol (TC) was estimated by colorimetric enzymatic estimation using the modified cholesterol oxidase/peroxidase method (Allan, 1974). Low-density lipoproteins (LDL-c) and very-low-density lipoproteins (VLDL-c) and chylomicrons were precipitated with sodium phosphotungstic acid magnesium chloride mixture and plasma HDL-c was measured enzymatically by modified cholesterol oxidase/peroxidase (Friedwald et al., 1972). Plasma triglycerides (TG) levels were measured enzymatically by the modified glycerol-3-phosphate oxidase /peroxidase method (Mcgowan, 1983). Measurements were performed using BT 1500 Fully Automated Biochemistry Analyzer (India) according to the manufacturer's instructions. The VLDL-c level was calculated by the formula: VLDL=TG÷5 and LDL-c level was calculated using Friedwald's formula =TC-[HDL+VLDL]) (Rifai et al., 2006).

5. Plasma levels of human sphingosine-1-phosphate (S1P) were measured with the enzyme-linked immunoassay (ELISA) kit (catalog no. MBS7254488, MyBioSource, Inc.,

California, San Diego, USA) by quantitative sandwich enzyme immunoassay technique. The intensity of color was measured spectrophotometrically at 450nm in a microplate reader and is inversely proportional to the S1P concentration (Aoki et al., 2005).

Statistical analysis

Obtained data were presented as mean, standard deviation, numbers, and percentages. Results were analyzed using One-way ANOVA for analysis of variance between groups and Chi-square test (X^2 test) for analysis of non-numeric data. Pearson's correlation analysis was applied to evaluate correlations between studied variables. The automatic linear modeling analysis was used to determine the importance of the variables for prediction of S1P, AST, and creatinine and HDL-c levels. Statistical analysis was conducted using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) for Windows statistical package. P value <0.05 was considered statistically significant.

RESULTS

During the visits for the villages included in health visits assigned by Benha University Hospital, 264 persons were eligible for evaluation; 136 had multiple exclusion criteria where 67 persons had chronic liver diseases, 41 persons had respiratory diseases, 34 persons had renal impairment of varying degrees and 27 persons were out of the age range. One hundred and twenty-eight persons fulfilled the inclusion criteria, but 15 of them were not inhabitants of the same village and so were not in continuous exposure and 5 refused to participate in the study, so 80 persons; 40 out

of each village were enrolled in the study (Fig. 1).

Ten urban volunteers of cross-matched age, sex, and body mass index, and free of exclusion criteria were included as a control group for laboratory investigations. The enrolment data are shown in table 1.
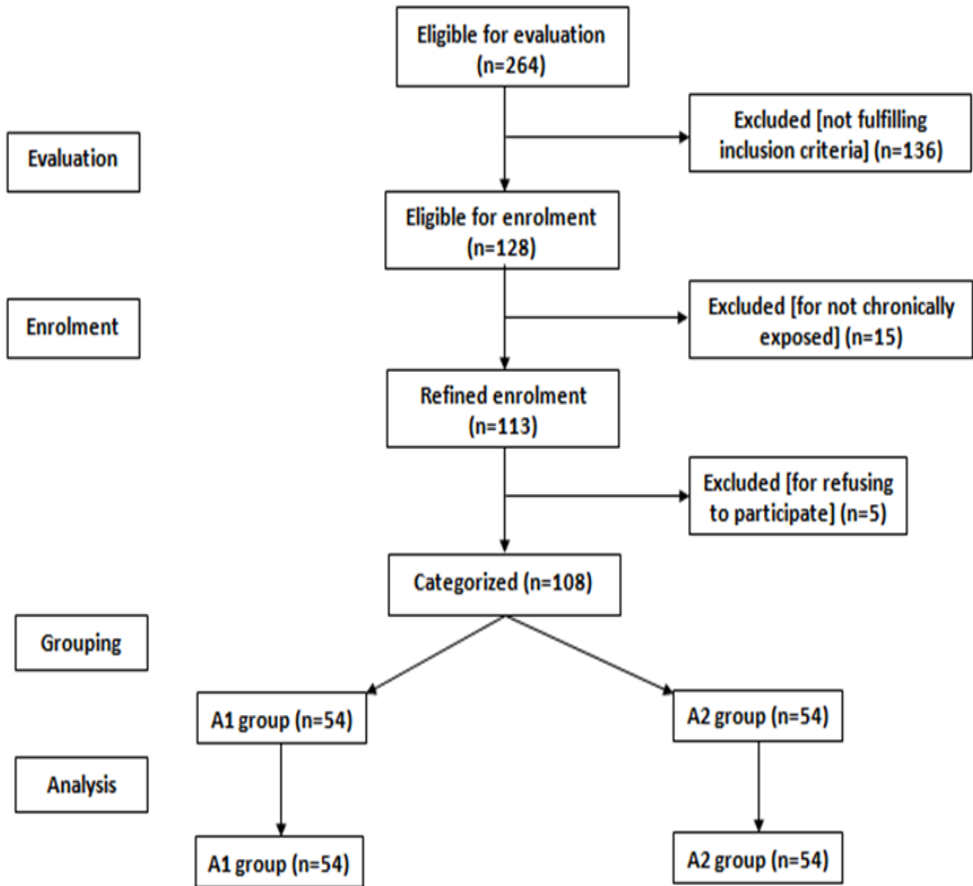

Figure 1: Consort Flow sheet

Table (1): Enrolment data of study participants

Variables		A1 Group (n=54)	A2 Group (n=54)	P-value
Age (years)	<25	6 (11.1%)	4 (7.4%)	0.285
	25-34	12 (22.2%)	14 (25.9%)	
	35-44	9 (16.7%)	17 (31.5%)	
	45-54	16 (29.6%)	9 (16.7%)	
	≥55	11 (20.4%)	10 (18.5%)	
	Mean (±SD)	42±11.8	40.9±11.2	0.624
Sex	Males	29 (53.7%)	24 (44.4%)	0.336
	Females	25 (46.3%)	30 (55.6%)	
Duration of exposure (years)	<5	21 (38.8%)	16 (29.6%)	0.461
	5-10	17 (31.5%)	14 (26%)	
	>10-20	11 (20.4%)	16 (29.6%)	
	>20	5 (9.3%)	8 (14.8%)	
	Mean (±SD)	9.3±7.7	10.3±7.2	0.513

Data are presented as mean & standard deviation (±SD); numbers & percentages; A1 group included participants collected from the area suspected to be heavily contaminated by fumonisins; A2 group included participants collected from the area suspected to be lightly contaminated by fumonisins; P-value indicates the significance of the difference between studied groups; P-value >0.05 indicates the non-significant difference between groups.

The mean levels of serum AST, ALT, and urea estimated in samples obtained from all the study participants were significantly higher in comparison to control levels, with significantly lower levels estimated in samples of participants of the A2 group in comparison to those of the A1 group. The mean serum creatinine levels estimated in samples of controls was lower than that of the study participant and the difference was significant versus levels estimated in samples of participants of the A1 group, but non-significant in comparison to levels estimated in samples of participants of A2 with significantly lower serum creatinine levels in samples of participants of A2 group than A1 group. Estimated plasma TC, LDL-c, and triglycerides levels showed non-significant differences between study samples and in comparison to control samples. However, plasma HDL-c levels estimated in control samples were significantly higher than levels estimated in samples of the study participants with significantly higher plasma HDL-c levels were estimated in samples of participants of the A2 group than in participants of the A1 group. Similarly, plasma S1P levels estimated in control samples were significantly higher than that estimated in samples of the participants of both the study groups with significantly higher levels in samples of participants of the A2 group in comparison to participants of the A1 group (Table 2, Fig. 2).

Table (2): Laboratory findings in samples collected from the study participants compared to levels estimated in samples of urban volunteers.

Variables	Control (n=10)	A1 Group (n=54)	A2 Group (n=54)	P1	P2	P3
RBG (mg/dl)	112.2±8.2	112±6.2	110±6.4	0.925	0.343	0.102
Hemoglobin conc. (gm/dl)	11.85±1	11.1±1.1	11.25±0.85	0.053	0.051	0.442
Serum AST (mg/ml)	22.2±3.3	31±6.8	28.1±7.4	0.001	0.016	0.039
Serum ALT (mg/ml)	24.3±2.7	34±7.5	29.5±6.7	0.001	0.019	0.0014
Serum urea (mg/ml)	26.4±4	33.9±6	31±6.7	0.042	0.003	0.021
Serum creatinine (mg/ml)	0.766±0.19	1.03±0.32	0.83±0.31	0.013	0.523	0.001
Total cholesterol (mg/dl)	188.3±10.8	183.5±18.7	185.6±16.5	0.622	0.436	0.535
HDL-c (mg/dl)	43.8±4	38±4.86	40±5.1	0.001	0.028	0.038
Triglycerides (mg/dl)	76.6±5.9	78.4±8.5	77.8±10.2	0.717	0.514	0.728
LDL-c(mg/dl)	67.9±8.9	67.1±21.5	67.8±15.9	0.903	0.984	0.839
Plasma S1P (nmol/L)	2481.9±787.7	1411±556.8	1761±823.3	<0.001	0.013	0.011

Data are presented as mean & standard deviation (±SD); A1 group included participants collected from the area suspected to be heavily contaminated by fumonisins; A2 group included participants collected from the area suspected to be lightly contaminated by fumonisins; RBG: Random blood glucose; AST: Aspartate transaminase; ALT: Alanine transaminase; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; S1P: Sphingosine-1-phosphate; P1 & P2 values indicate the significance of the differences between A1 & A2 groups, respectively and control levels; P3 value indicates the significance of the difference between A1 & A2 groups; P-value <0.05 indicates the significant difference; P-value >0.05 indicates the non-significant difference.
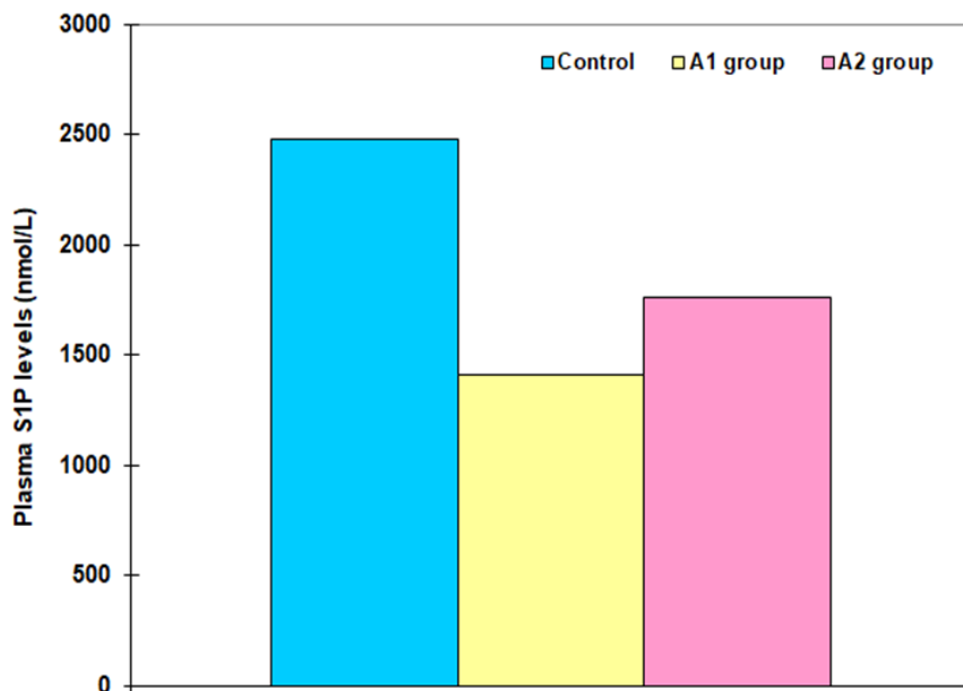
Fig. (2): Plasma S1P levels estimated in study participants and controls

Estimated serum AST, ALT, urea and creatinine, and plasma triglycerides showed a positive significant correlation with the duration of exposure, while plasma HDL-c showed a negative significant correlation with the duration of exposure. Estimated plasma S1P levels showed a negative significant correlation with participants' age (Fig. 3), duration of exposure (Fig. 4), and serum levels of AST, ALT, urea, and creatinine, while showing a positive significant correlation with plasma levels of HDL-c (Table 3).

Table (3): Pearson's correlation between duration of exposure to maze and plasma S1P levels and demographic and laboratory data of study participants.

Variables	Duration of exposure		S1P level	
	r	p	r	p
Age	0.807	<0.001	-0.792	<0.001
Male gender	0.076	0.437	-0.093	0.339
Duration of exposure	-	-	-0.779	<0.001
RBG	-0.007	0.942	-0.116	0.231
Hb. Conc.	-0.101	0.299	0.151	0.120
S. AST level	0.873	<0.001	-0.857	<0.001
S. ALT level	0.822	<0.001	-0.853	<0.001
S. Urea level	0.749	<0.001	-0.801	<0.001
S. Creatinine level	0.639	<0.001	-0.759	<0.001
Total cholesterol level	0.035	0.718	0.043	0.661
HDL-c level	-0.775	<0.001	0.852	<0.001
Triglyceride level	0.194	0.045	-0.188	0.052
LDL-c level	0.144	0.136	-0.095	0.328

Data are presented as Pearson's correlation coefficient "r"; RBG: Random blood glucose; Hb. conc.: Hemoglobin concentration; AST: Aspartate transaminase; ALT: Alanine transaminase; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; S1P: Sphingosine -1-phosphate; P-value indicates significance of the coefficient; P-value <0.05 indicates significant; P-value >0.05 indicates non-significant correlation.
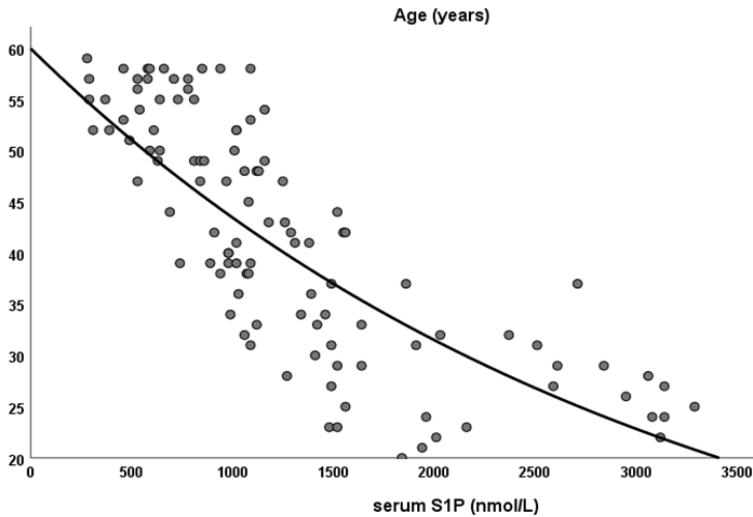

Figure (3): Pearson's correlation between estimated plasma S1P levels and participants' age
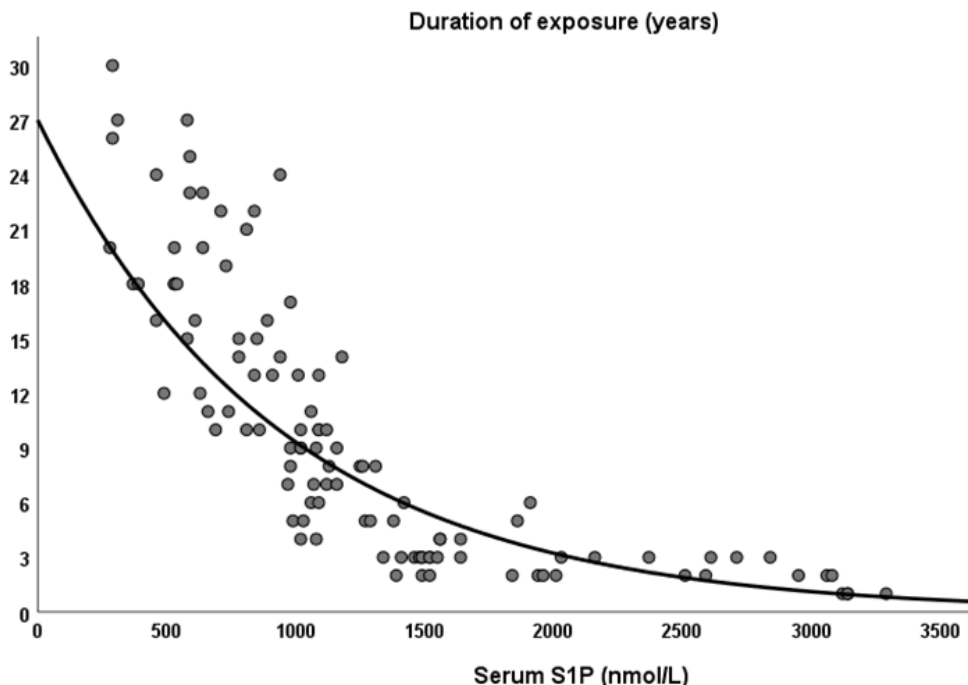
Duration of exposure (years)

Figure (4): Pearson's correlation between estimated plasma S1P levels and duration of exposure to maze plant.

The automatic linear modeling regression analysis for the importance of participants' demographic data and lab findings as predictors for low plasma S1P are long duration and severity of exposure to maze plant and older age (Fig. 5); for liver affection as manifested by high serum AST are duration and severity of exposure to maze plant, older age, low plasma S1P levels and male gender (Fig. 6), and for kidney affection, as manifested by high serum creatinine are low plasma S1P, severity, and duration of exposure to maze plant (Fig. 7). The important predictors for low plasma HDL-c as a marker for the possibility of oncoming cardiac insults are low serum levels of S1P, duration, and severity of exposure to maze plant (Table 4, Fig. 8).

Table (4): The automatic linear modeling regression analysis for the importance of demographic data and lab findings as predictors for low plasma S1P, high serum AST and creatinine, and low plasma HDL-c levels.

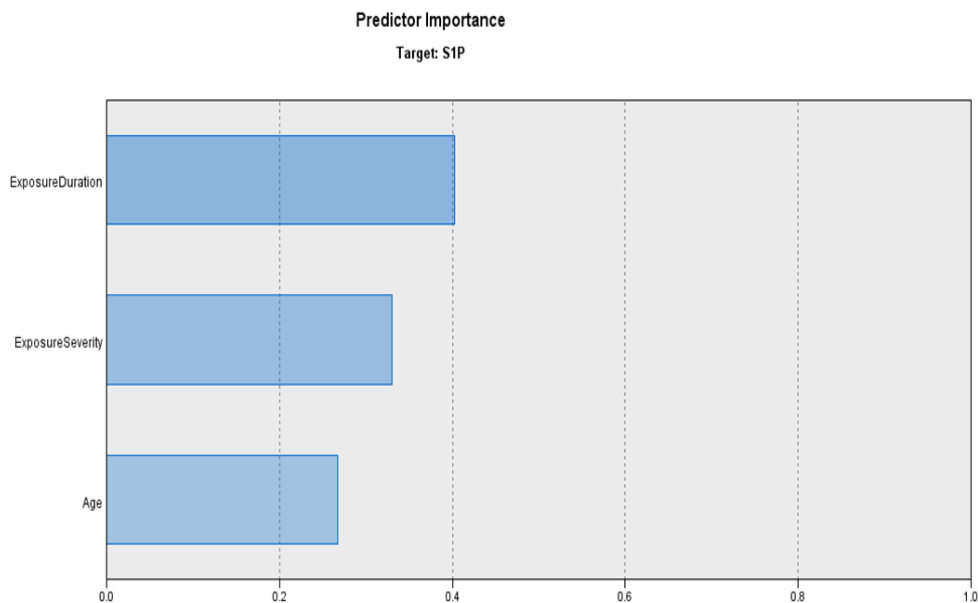| Variables	Study target			
	Low plasma S1P	High serum AST	High serum creatinine	Low plasma HDL-c
	Importance of variable as a predictor for the target			
Age	27%	17%	Excluded	Excluded
Male gender	Excluded	4%	Excluded	Excluded
Heavy exposure to maze	33%	16%	22%	4%
Duration of exposure	40%	53%	14%	27%
Plasma S1P level	-	10%	64%	69%

Predictor Importance

Target: S1P

Figure (5): The automatic linear modeling regression analysis for the importance of studied variables as predictors for low plasma S1P levels.
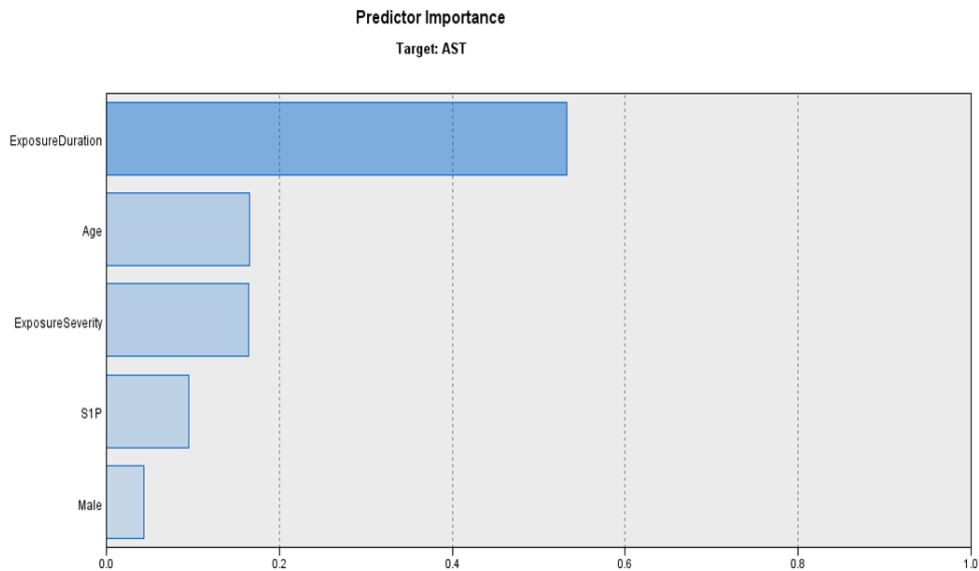
Predictor Importance

Target: AST

Figure (6): The automatic linear modeling regression analysis for the importance of studied variables as predictors for high serum AST levels as a measure for liver function impairment.
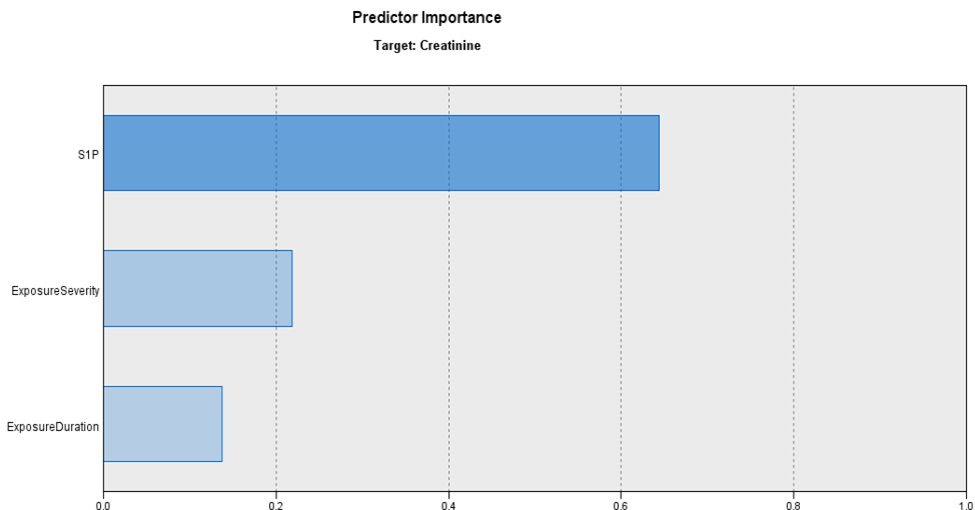
Predictor Importance

Target: Creatinine

Figure (7): The automatic linear modeling regression analysis for the importance of studied variables as predictors for high serum creatinine levels as a measure for kidney function impairment.
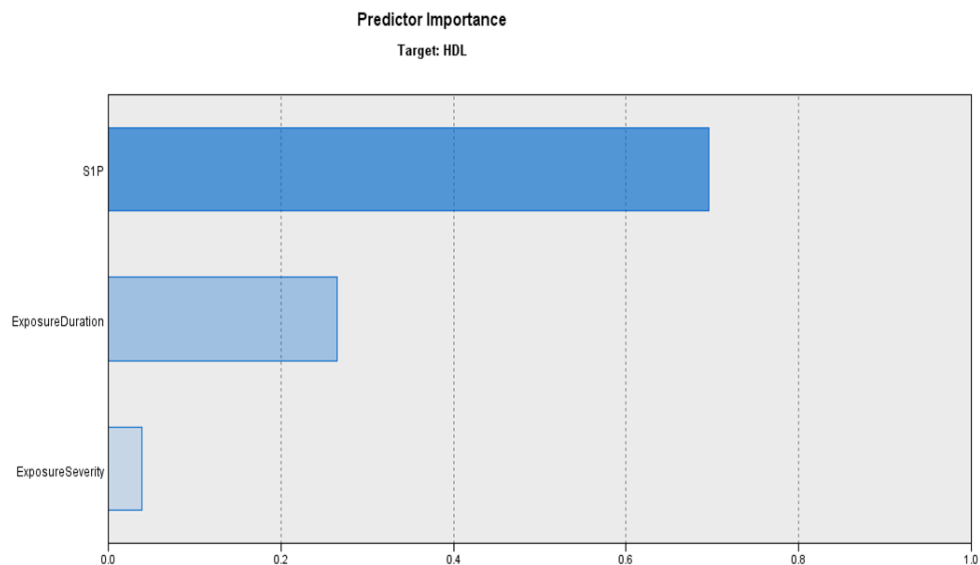
Figure (8): The automatic linear modeling regression analysis for the importance of studied variables as predictors for low plasma HDL-c levels as a measure for risk of cardiac insults.

DISCUSSION

Exposure to fumonisins through contact with maze plants or during manipulations of its out products deleteriously affected liver and kidney functions of farmers or workers as manifested by elevated random levels of liver enzymes and kidney function tests in comparison to levels estimated in volunteers who denied contact with maize or it's our products.

These findings assured the results of the early animal studies that detected centrilobular hypertrophy and cytoplasmic vacuolization in hepatic sections obtained from animals exposed to fumonisin with increased hepatic binucleated cells and acidophilic body, and kidney lesions which were consistent with tubular nephrosis, with tubular dilatation and cell debris (Kim et al., 2006). Additionally, another study detected similar severe histological and histochemical changes in liver and kidney tissues with increased serum levels of ALT, AST, creatinine, and uric acid in animals that received fumonisins (El-Nekeety et al., 2007). Thereafter, Singh & Kang (2017) using an animal model of chronic fumonisin exposure detected altered levels of hepatic enzymes and induced histopathological changes with increased expression levels of major endoplasmic reticulum stress and autophagy-related markers such as PERK, IRE1-α, and LC3I/II.

The current study detected significantly lower serum sphingosine-1-phosphate (S1P) in samples of participants in both A1 and A2 groups in comparison to levels estimated in volunteers

with significantly lower levels in samples of participants of the area of heavy contamination. Similarly, Grenier et al., (2012) using two animal models of fumonisin toxicity received hydrolyzed or non-hydrolyzed fumonisins detected low hepatic and intestinal toxicity of hydrolyzed fumonisin that correlated with a weak alteration of the sphinganine/sphingosine ratio in the liver and the plasma in comparison to animals received non-hydrolyzed fumonisins.

These findings spotlight the effect of fumonisins on phospholipid metabolism in direction of impaired ceramide synthesis with subsequent altered cellular metabolism, cell wall integrity, and cellular functions. Furthermore, there was a negative significant correlation between serum levels of S1P and serum levels of AST, ALT, creatinine, and urea, thus pointing to the possibility of considering liver and kidney as the target tissues for fumonisin toxicity.

In support of the toxic effect of fumonisin on hepatocyte and renal tubular cells, there was a positive significant correlation between serum levels of liver enzymes and renal excretory products with duration of exposure to fumonisins. In line with these findings, Harrer et al., (2015) using an animal model of chronic fumonisin exposure detected N-acyl derivatives of fumonisin B1 in liver and kidney with variable length of N-acyl chain in a tissue-dependent manner with C16 and C24 derivatives in the kidney and the liver, respectively.

In a trial to explore the underlying mechanisms for fumonisin toxicity, Abbès et al., (2016) detected DNA damage and cellular death in tissues exposed to fumonisins and attributed this to induction of oxidative stress and immunotoxicity on fumonisin exposure leading to an increase in caspase-3 activity which causes DNA damage and cellular death. Also, Singh & Kang (2017) considered fumonisin toxicity was related to the inhibition of ceramide synthase leading to the accumulation of sphingoid bases, which in turn cause the development of oxidative stress.

As another mechanism, Gardner et al., (2016) and Yamazoe et al., (2017) attributed the toxic effects of fumonisin to disruption of phospholipid metabolism through the accumulation of sphingosine kinase-2 which is predominantly present in the nucleus leading to increased nuclear sphingosine-1-phosphate in the nucleus leading to inhibition of histone deacetylase activity with subsequently increased acetylation of histone lysine residues and failure of DNA supercoiling and fragmentation.

Statistical analysis using the Automated Linear Modeling regression analysis defined low S1P serum levels as a significant predictor for impaired liver and kidney function despite being healthy individuals and so could be used as an early predictor of these dysfunctions before clinically manifest or more complicated. These findings supported that detected by early animals studies that documented that disruption of sphingolipid metabolism is an early and sensitive biomarker of fumonisin exposure (Tardieu et al., 2007).

CONCLUSION

Chronic fumonisin exposure through dealing with the maze or its by-products deleteriously, but gradually affects kidney and liver functions. Such

effect may be direct or through disturbing the sphingolipid metabolism with suppression of ceramide synthesis and depletion of sphingosine. Low serum levels of S1P were found to correlate with renal and hepatic affection and could be used as an early marker for the detection of such changes.

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اضطراب مستويات السفينجوزين – ١ فوسفات بمصل الدم يُمكن من التنبؤ بوظائف الكبد والكلى المضطربة في الأشخاص الأصحاء المعرضين للفومونيزين بشكل مزمن

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الاهداف: تحاول الدراسة الحالية تحديد تأثير الفومونيزين على الكلى واختبارات وظائف الكبد وعلى مستقبلات الدهون السفينجولية.

المرضى والوسائل: ٨٠ متطوع صحيين بوضوح، ٤٠ من المنطقة ١ والتي تعد من المناطق شديدة التلوث بالفومونيزينات والمنطقة ٢ التي تعد منطقة أقل تلوثا بالفومونيزينات. التحق عشر متطوعين من المناطق الحضارية كمجموعة التحكم. خضع كل المشتركين لفحص سريري كامل خاصة للكبد والكلى وتم اخذ عينات دم من اجل تحديد ومعدلات ناقلات الاسبراتين (AST), ناقلات الألانين (ALT), اليوريا, الكرياتينين ومعدلات الدهون ببلازما الدم وتحديد ومعدل السفينجوسين–١–فوسفات (S١P) باستخدام تقنية الإليزا.

النتائج: كانت معدلات AST, ALT واليوريا والكرياتنين مرتفعة بشكل واضح بمصل الدم, بينما كانت معدلات البروتينات الدهنية عالية الكثافة و (S١P) كانت اقل بشكل واضح في عينات المشاركين بالمقارنة بعينات مجموعة التحكم, مع اختلاف واضح بين المشاركين من كلا المجموعتين. اظهرت فترة التعرض للفومونيزينات علاقة طردية مع معدلات AST, ALT و اليوريا والكرياتنين, بينما اظهرت علاقة عكسية مع معدلات البروتينات الدهنية عالية الكثافة، S١P في البلازما. اظهرت معدلات S١P علاقة عكسية مع اعمار المشاركين ومعدلات AST, ALT و اليوريا و الكرياتنين، بينما اظهرت علاقة طردية مع معدلات البروتينات الدهنية عالية الكثافة بالبلازما. حدد

التحليل المرجعي ان التعرض للفومونيزينات لفترة طويلة وشدة التعرض وتقدم العمـــر كمؤشرات لنقص S١P في البلازما والذي يعتبر مؤشر لخلل في نسب انزيمات الكبـد, وتأثر الكلى, و نقص البروتينات الدهنية عالية الكثافة في البلازما.

الاستنتاج:التعرض المزمن للفومونيزينات يشكل ضرر, و يؤثر بالتدريج على وظائف الكلى والكبد بشكل مباشر او عن طريق احداث خلل في التمثيـــل الغـــذائي للـــدهون السفينجولية واستنزاف السفينجوسين. اتضح ان نسبة ال S١P في الدم تتناسب مع التأثر الكلوي والكبدي ومن الممكن استخدامها كمؤشر مبكر للتحقق من هذه التغيرات.

٢٥. المجلة المصرية للعلوم الطبية ٣٩(٢) ديسمبر ٢٠١٨ : ٩٤١–٩٥٩.