Plastic toxicity effects on Egyptian workers

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ABSTRACT

The basic structure of plastics (or polymers) is given by macromolecule chains, formulated from monomer units by chemical reactions, such: Poly vinyl chloride (PVC), Vinyl chloride monomer (VCM), Bisphenol A (BPA) and Polypropylene (PP), depending on the type of bonding partners. The aim of the present study was to investigate the effect of plastic on Egyptian workers who had undergone for liver function analysis, (AST and ALT), and (SERPINA1 gene) which provides instructions for making a protein called alpha-1 antitrypsin (A1AT), which is a type of serine protease inhibitor (serpin). One hundred male participants from Qluobiya Governorate were included in this study. They were divided into two groups, 50 healthy participants with no history of plastic exposure (control), and 50 workers occupationally exposed to plastic. Liver functions of both groups (AST & ALT) were determined spectrophotometrically, also liver viruses were analysed for all populations (control and exposer), and the result was negative for all. The two groups were compared as regards to genotype results in a version of the (SERPINA1 gene), they are S and Z allele genotypes that produces alpha-1 antitrypsin using ARMS PCR (Amplification-Refractory Mutation System). The results showed Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), were significantly ($p<0.05$) elevated in the exposed group compared to control, and The mean of both AST & ALT were significantly increased ($P<0.001$) in exposed individuals with MS genotype than control. Heterozygous (MZ), (SZ) and homozygous (SS), (ZZ) genotypes were significantly more frequent in exposed than in controls ($P<0.01$).

Key words: *Serpin A1, A1AT, Plastic, Egyptian plastic workers, ALT, AST*

INTODUCTION

All plastic waste management technologies (including incineration, co-incineration, gasification, and pyrolysis) are harmful. this is due to releasing of toxic metals such as lead and mercury, organic substances (dioxins and furans), acid gases, and other toxic substances to the air, water, and soils. All such technologies lead to direct and indirect exposure to toxic substances for workers and nearby communities, that can be occurred through inhalation of contaminated air, direct contact with contaminated soil or water, and ingestion of foods that were grown in an environment polluted with these substances. Toxins from emissions, fly ash, and slag in a burn pile can travel long distances and deposit in soil and water, eventually entering human bodies after being accumulated in the tissues of plants and animals **(Caron, 2017).**

Microfibers and other plastic micro particles also are increasingly being documented in human tissues. Until these impacts are better understood, we should adopt a precautionary approach to limit the production and use of these persistent contaminants and that indicated by **(Carbery et al., 2018).**

The Alpha-one antitrypsin molecule is produced mainly in the liver and, in smaller quantities, in macrophages and in the bronchial epithelium. Through the circulation, AAT reaches the lungs, where it will perform its anti-elastolytic function. AAT is also acute phase protein **(Perlmitter, 2007).**

Based on serum levels of AAT and molecular function in combination with allelic variants, these are classified into four groups: normal (M alleles), deficient (Z, S alleles), dysfunctional (normal serum AAT but with reduced function, such as (F and Pittsburgh alleles) and null (undetectable serum level of AAT, QO alleles) which produce premature stop codons **(Topic et al., 2002).** AAT deficiency disorders include neonatal cholestasis, liver cirrhosis, and emphysema in the third to fifth decade of life, and panniculitis **(Lomas and Parfrey, 2004).**

The glycoprotein AAT is encoded in the SERPINA1 gene (Serine protease inhibitor A1), This gene consists of one untranslated exon followed by four translated exons **(de Serres et al., 2014).**

AST levels rise 10 to 20 **times** than normal. On the other hand, the ratio of AST to ALT (AST/ALT) sometimes can help determine whether the liver or another organ has been damaged **(Suman S et al., 2005)** DNA damage has also been related to plastic exposure. Plastics can interact with DNA leading to infertility **(Helmestam et al., 2014),** degenerative diseases **(Malaguarnera et al., 2011)** and ultimately to cancer (*Dragani et al., 2008*). In addition, studies showed that exposure to plastic components could induce, chromosomal aberrations (CA), sister chromatid exchange (SCE), micronuclei frequency (MN), genotoxicity, hepatocarcinogenesis. Also oxidative stress such as lipid oxidation **(Bolt, 2005), (Dogliotti, 2006) (Cheong et al., 2016), (Ferreira et al., 2015), (Laing et al., 2016).**

Alpha-one antitrypsin (AAT) is glycoprotein and member of serine protease inhibitor (Pi) family and its principal function is to inhibit a series of enzymes, among which are trypsin[1], elastase[2], and protease-3[3].

Consisting of 50 workers occupationally exposed to plastic materials. Individuals suffering from diabetes, hypertension, hypercholesterolemia, liver and kidney dysfunctions, and any other chronic diseases not related to plastic materials exposure were excluded from the study.

Body Mass Index (BMI)

Portable mechanical analog scales were used to measure height and weight, BMI calculated by the following formula.

BMI= weight (Kg) / Height2 (meter2)

Blood Collection

Five ml of venous blood were collected, 2ml in Ethylene Diamine Tetraacetic acid (EDTA- K3) sterile labeled tubes and 3 ml in sterile labeled tubes. EDTA tubes were centrifuged at 1000 rpm for 10 minutes, Plasma was collected and EDTA blood stored at -20°C for subsequent DNA extraction. 3 ml of blood was collected into a sterile labeled tube and allowed to clot, and then the blood was centrifuged at 3000 rpm for 10 minutes.

The serum was removed for biochemical analysis in the same day.

Over 75 allelic variants have been reported and classified using the protease inhibitor (Pi) nomenclature that assesses AAT mobility in isoelectric focusing analysis **(Mayer et al., 2015).**

Patients exhibiting two AAT deficiency alleles are generally considered to be at risk, whereas patients with a single AAT deficiency allele are considered to be at low risk for the development of AAT deficiency- related diseases. Plastic which manufactured in the factory by workers can cause liver damage and in this study we intend to assess the effects of exposure to plastic materials on liver by determining AST & ALT enzymes which reflect the case of liver and how liver affected by those materials **(Hsiao et al., 2004).**

MATERIALS AND METHODS

Study Population

One hundred male participants from seven factories in Qaluob, Qluobiya Governorate, Egypt were included in this study. They were divided into two groups: - Group I: control group consisting of 50 healthy participant with no history of occupational plastic exposure. - Group II

Procedure

Samples and reagents were brought to room temperature prior to the analysis, 1ml of working reagent incubated for 5 minute at 37ºC, running normal and pathological high control. The spectrophotometer was programmed for wavelength 340 nm, 37ºC and 500 µL aspiration volume.

100 µL of serum added to 1.0 ml of working reagent and immediately aspirated by spectrophotometer that incubates at 37ºC for one minute. Based on the intensity of color compound formed, it reads the absorbance kinetically at one, two, three minutes to determine the activity of ALT in the sample by calculating the average change in absorbance per minute.

●Calculations

ΔA/min x 1750 = U/L of ALT where ΔA = average change

●Normal values for adult male

Up to 40 IU/L

Biochemical Analyses

Biochemical Analyses were performed by ERMA AE-600N biochemical analyzer (ERMA INC. Japan) following standard procedures for clinical biochemistry purposes. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) as hepatotoxicity markers.

a) Determination of Serum Alaninamino-transferase (ALT)

● Principle

ALT catalyzes the transfer of the amino group from alanine to α-ketoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of

NADH to NAD+ through the activity of ALT present in the sample.

Reagent Composition and preparation

The kit content of ALT supplied from SPINREACT (Spin) are presented in (table 1), Working reagent (WR) prepared by mixing 800 µL of R1 + 200 µL of R2.

100 µL of serum added to 1.0 ml of working reagent and immediately aspirated by spectrophotometer that incubates at 37ºC for one minute. Based on the intensity of color compound formed, it reads the absorbance kinetically at one, two, three minutes to determine the activity of ALT in the sample by calculating the average change in absorbance per minute.

●Calculations

ΔA/min x 1750 = U/L of AST where ΔA = average change

●Units

The concentration is expressed in units per liter of sample (U/L). One international unit (IU) is the amount of enzyme that transforms 1.0 µmol of substrate per minute, in standard conditions.

●Normal values for adult male

Up to 38 IU/L

b) Determination of Serum Aspartate Aminotransferase (AST)

●Principle

AST catalyzes the transfer of the amino group from asparate to α-ketoglutarate with the formation of glutamate and oxaloacetate. The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+ proportional to the activity of AST present in the sample.

●Reagent Composition and Preparation

The kit content of AST supplied from SPINREACT (Spain) are presented in (Table 2), Working reagent (WR) prepared by mixing 800 µL of R1 + 200 µL of R2.

●Procedure

Samples and reagents were brought to room temperature prior to the analysis, 1ml of working reagent incubated for 5 minute at 37ºC, running normal and pathological high control. The spectrophotometer was programmed for wavelength 340 nm, 37ºC and 500 µL aspiration volume.

Table 1. Contents of ALT kit.

Item	Content	Concentration
R1 Buffer	**TRIS pH 7.8** **Lactate dehydrogenase(LDH)** **L-Alanine**	**100 mmol/L** **1200 U/L** **500 mmol/L**
R2 Substrate	**NADH** **α-Ketoglutarate**	**0.18 mmol/L** **15 mmol/L**

Table 2. Contents of AST kit.

Item	Content	Concentration
R1 Buffer	**TRIS pH 7,8** **Lactate dehydrogenase (LDH)** **Malate dehydrogenase (MDH)** **L-Aspartate**	**80 mmol/L** **800 U/L** **600 U/L** **200 mmol/L**
R2 Substrate	**NADH** **α-Ketoglutarate**	**0.18 mmol/L** **12 mmol/L**

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 19 (LEAD Technology Inc) and (EXCEL 2016). Data were presented as means with corresponding standard deviation (SD). Each polymorphism was examined in the control population to confirm that the distribution of the genotypes confirmed to Hardy–Weinberg expectations (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).Group II (**exposed**):50 plastic workers occupationally exposed to plastic and did not use any protective equipment during work hours, their age ranged between 20– 60 years (mean ± SD 37.4±9.70). Demographic characteristic of the two groups revealed that there was highly significant (P<0.01) increase in age among exposed group compared to control group, Tables (5,6) and Figures (3,4) shows the Ages data in the fifty persons in both Control and Exposed groups.

The mean of (Age) that represented exposed group was higher than the control and that significant statistically (P < 0.01), but in case of BMI in both groups there was no significant difference in the body mass index (BMI) in the two groups. Table (7) & Figure (5).

4.3. Biochemical Analyses Results

The biochemical analyses were done for 100 persons from Qluobiya city, Egypt. Of whom 50 control and 50 exposed to plastic. ALT and AST were measured spectrophotometrically.

a) Alani aminotransferase (ALT).

ALT was measured by kinetic methods; the normal range for adult male is (10 - 40 IU/L). The results revealed that ALT activity in the control group

RESULTS

This study was conducted on 100 Persons from Qaluob, Qluobiya government, Egypt. During the period from April 2016 to October 2017.

BMI

The studied population was classified into two groups. Group I (**control**): 50 apparently healthy males with no history of occupational plastic exposures, Group II (**exposed**):50 plastic workers occupationally exposed to plastic and did not use any protective equipment during work hours, there was no significant difference in the body mass index (**BMI**) in the two groups.

Table (3) and Figure (1) show the BMI data in the fifty persons in both Control and Exposed group with the calculated: Mean, Standard deviation, Frequency and Percentage. Table (4) and figure (2).

Age

The studied population was classified into two groups. Group I (**control**): 50 apparently healthy males with no history of occupational plastic exposures, their **age**

ranged between 20 – 60 years (mean ± SD 33.24±9.06).

(mean ± SD 24.16±6.44) IU/L, while ALT activity of exposed group (mean ± SD 28.80±7.85) IU/L, tables (8, 9) & figures (6, 7).

b) Aspartate aminotransferase (AST).

AST was measured by kinetic methods; the normal range for adult male is (10-38 IU/L) The results revealed that AST activity in the control group (mean ± SD 28.56±7.69) IU/L, while AST activity in the exposed group (mean ± SD 35.38±12.04) IU/L, tables (10, 11) & figures (8, 9).

The effect of occupational exposure to plastic on ALT and AST as hepatotoxicity markers are shown in (Table 12 & Fig 10). Highly significant (P<0.05) increase in ALT and AST among exposed group was observed compared to control group.

Table 4. Frequency and Percent for BMI of exposed group

BMI		Frequency	Percent %
Exposed	18	1	2.0
	19	1	2.0
	20	1	2.0
	21	5	10.0
	22	10	20.0
	23	8	16.0
	24	11	22.0
	25	8	16.0
	26	2	4.0
	27	1	2.0
	28	2	4.0
	Total	50	100.0

Table 3. Frequency and Percent for BMI of control group

BMI		Frequency	Percent %
Control	18	4	8.0
	19	4	8.0
	20	1	2.0
	21	5	10.0
	22	7	14.0
	23	7	14.0
	24	10	20.0
	25	7	14.0
	26	2	4.0
	27	1	2.0
	28	2	4.0
	Total	50	100.0

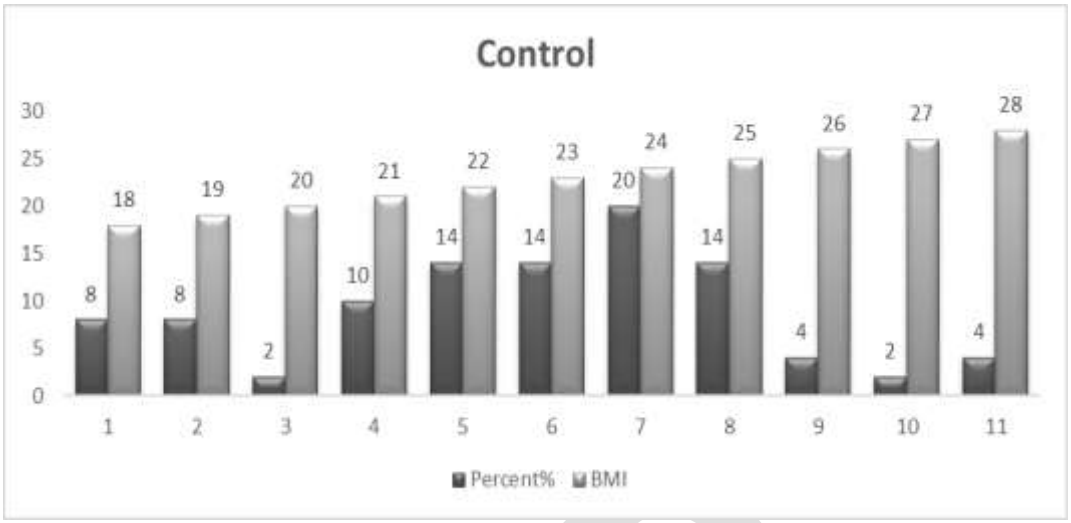

Fig (1). Percentage of higher and lower BMI for control group.

In control cases, the higher percentage was for the normal BMI, but the lower percentage was for overweight BMI.

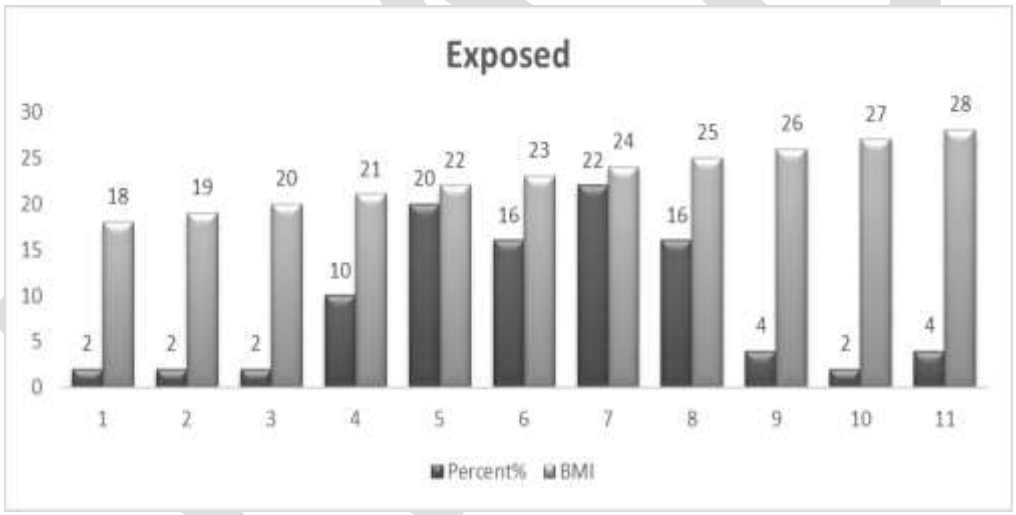

Fig (2). Percentage of higher and lower BMI for exposed group**.**

In exposed cases, the higher percentage was for the normal BMI but, the lower percentage was for the overweight BMI.

Table 5. Age of control group

Age		Frequency	Percent %
Exposed	20	3	6.0
	24	1	2.0
	25	3	6.0
	27	2	4.0
	29	3	6.0
	30	1	2.0
	32	2	4.0
	33	1	2.0
	34	4	8.0
	36	2	4.0
	37	4	8.0
	38	2	4.0
	39	1	2.0
	40	3	6.0
	41	2	4.0
	42	3	6.0
	44	3	6.0
	45	2	4.0
	46	1	2.0
	47	1	2.0
	48	1	2.0
	53	2	4.0
	55	1	2.0
	60	2	4.0
	Total	50	100.0

Table 6. Age of exposed group

Age		Frequency	Percent %
Control	20	4	8.0
	21	3	6.0
	22	1	2.0
	23	1	2.0
	25	1	2.0
	26	2	4.0
	27	2	4.0
	28	2	4.0
	29	4	8.0
	30	2	4.0
	31	1	2.0
	33	2	4.0
	34	2	4.0
	36	2	4.0
	37	6	12.0
	38	2	4.0
	39	1	2.0
	40	4	8.0
	41	2	4.0
	43	1	2.0
	44	1	2.0
	45	1	2.0
	48	1	2.0
	55	1	2.0
	60	1	2.0
	Total	50	100.0

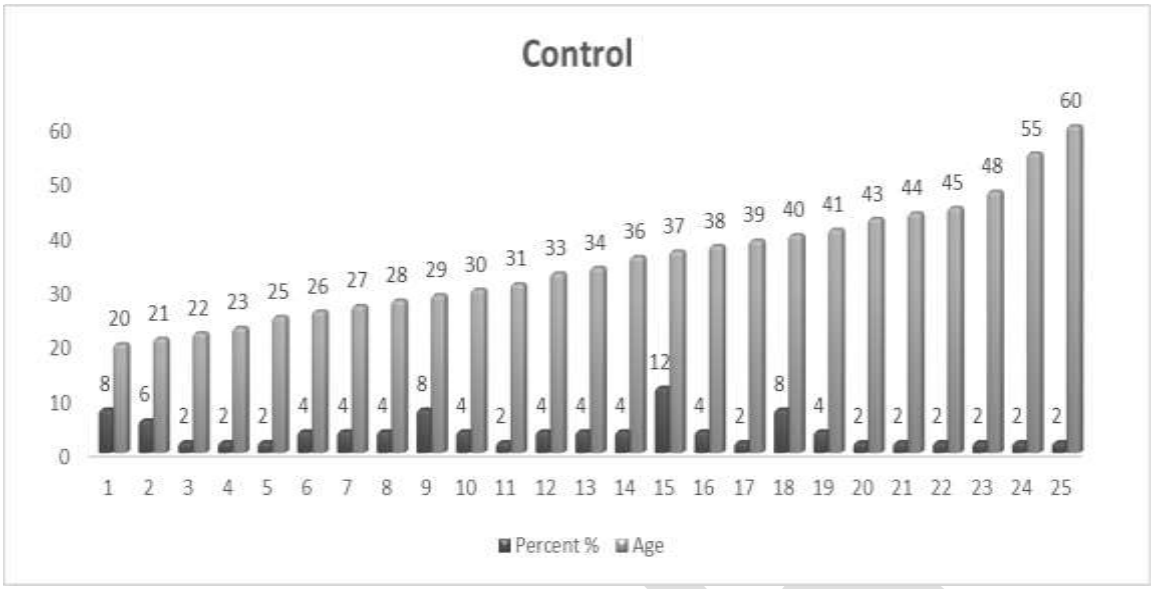

Fig (3). Percentage of higher and lower ages for control group.

In control cases, the higher percentage was for the medium ages, but the lower percentage was for the higher ages.

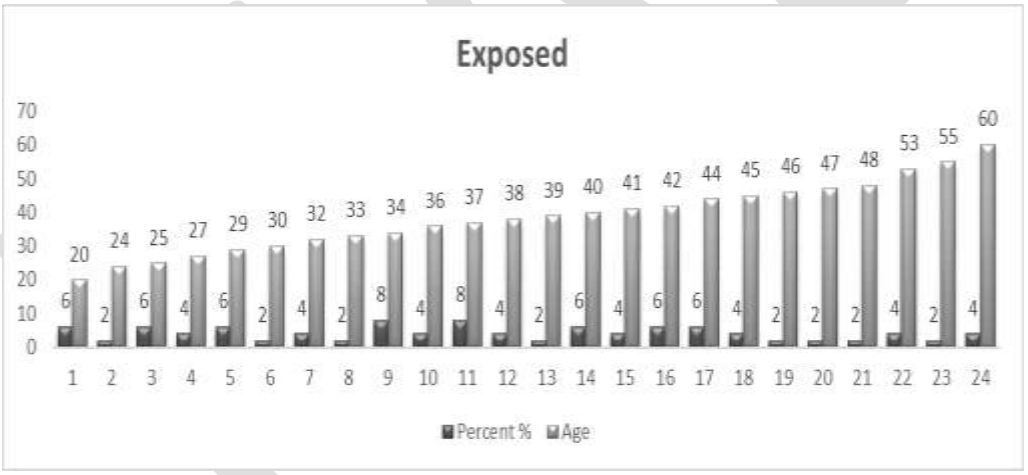

Fig (4). Percentage of higher and lower ages for exposed group.

In exposed cases, the higher percentage was for the medium ages such, but the lower percentage was for the higher ages.

Table 7. Age and BMI for control and exposed group.

Parameter	Control (N=50)	Exposed (N=50)	P value	Correlation
Age (years)	**33.24±9.06**	**37.4±9.70**	**<0.01**	**0.368**
BMI (kg/m2)	**22.76±2.55**	**23.30±2.03**	**NS**	**_____**

Data represented as mean ± standard deviation (SD), N= number, P < 0.01 significant

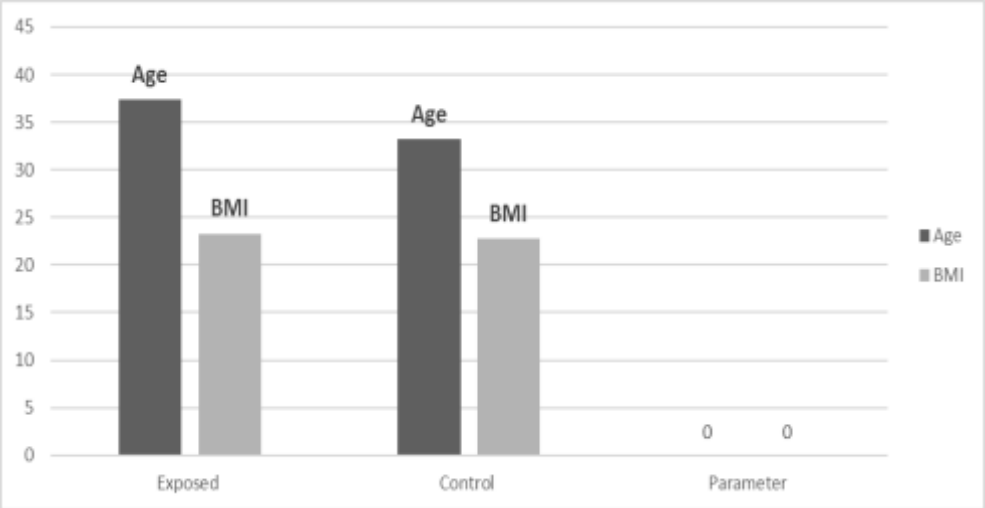

Fig. 5. Comparison between mean age and BMI in control and exposed group.

Table 8. Frequency and Percent for ALT of exposed group

ALT IU/L		Frequency	Percent %
Exposed	**15**	**1**	**2.0**
	16	**4**	**8.0**
	18	**2**	**4.0**
	20	**1**	**2.0**
	21	**4**	**8.0**
	22	**3**	**6.0**
	23	**1**	**2.0**
	25	**2**	**4.0**
	26	**1**	**2.0**
	27	**2**	**4.0**
	28	**3**	**6.0**
	30	**4**	**8.0**
	31	**1**	**2.0**
	32	**2**	**4.0**
	34	**2**	**4.0**
	35	**5**	**10.0**
	36	**1**	**2.0**
	37	**3**	**6.0**
	38	**4**	**8.0**
	39	**2**	**4.0**
	40	**1**	**2.0**
	43	**1**	**2.0**
	Total	**50**	**100.0**

Table 9. Frequency and Percent for ALT of control group

ALT IU/L		Frequency	Percent %
Control	**14**	**1**	**2.0**
	16	**5**	**10.0**
	18	**8**	**16.0**
	19	**1**	**2.0**
	21	**5**	**10.0**
	22	**6**	**12.0**
	25	**5**	**10.0**
	26	**3**	**6.0**
	27	**1**	**2.0**
	28	**4**	**8.0**
	29	**1**	**2.0**
	30	**2**	**4.0**
	34	**1**	**2.0**
	35	**5**	**10.0**
	36	**1**	**2.0**
	38	**1**	**2.0**
	Total	**50**	**100.0**

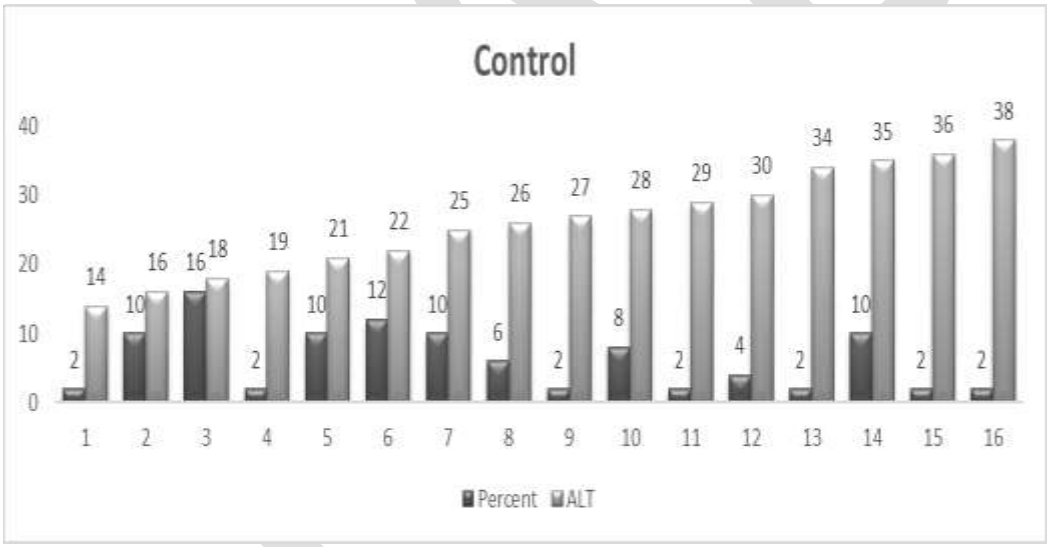

Fig. 6. Percentage of ALT results for control group.

The highest percentage of ALT was for the cases who their ALT was 16 IU/L, the lower percentage of ALT was for the cases who their ALT ranged from 34-38 IU/L.

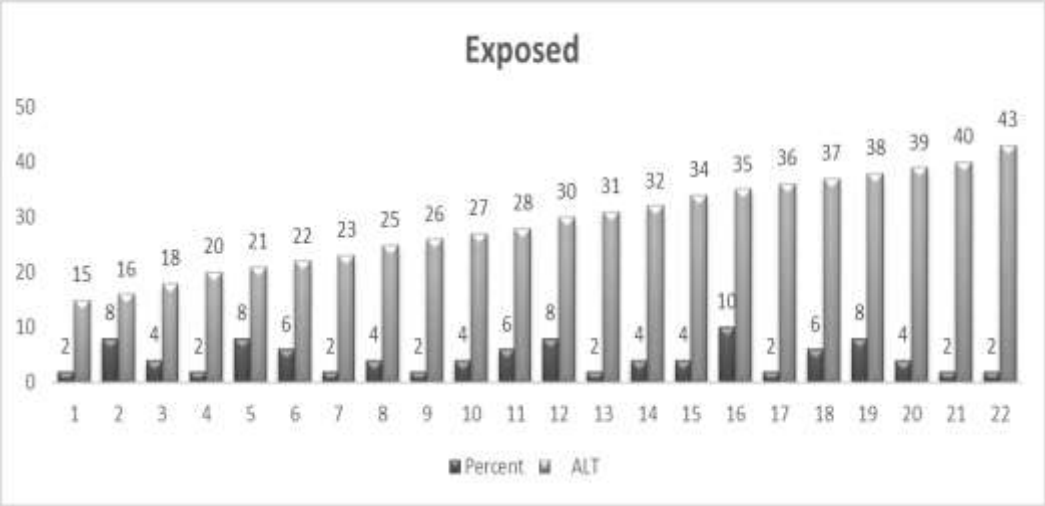

Fig. 7. Percentage of ALT results for exposed group.

The higher percentage of ALT was for the cases who their ALT was 16, 21, 35 IU/L, the lower percentage of ALT was for the cases who their ALT ranged from 20, 23, 40, 43, IU/L.

Table 10. Frequency and Percent for AST of control group.

AST IU/L		Frequency	Percent %
Exposed	**17**	**1**	**2.0**
	18	**3**	**6.0**
	19	**3**	**6.0**
	25	**6**	**12.0**
	27	**2**	**4.0**
	29	**1**	**2.0**
	30	**1**	**2.0**
	34	**3**	**6.0**
	35	**2**	**4.0**
	36	**2**	**4.0**
	37	**5**	**10.0**
	38	**1**	**2.0**
	39	**1**	**2.0**
	40	**3**	**6.0**
	41	**3**	**6.0**
	42	**4**	**8.0**
	43	**2**	**4.0**
	45	**3**	**6.0**
	48	**1**	**2.0**
	49	**1**	**2.0**
	56	**1**	**2.0**
	87	**1**	**2.0**
	Total	**50**	**100.0**

Table 11. Frequency and Percent for AST of control group.

AST IU/L		Frequency	Percent %
Control	17	1	2.0
	18	6	12.0
	20	1	2.0
	21	4	8.0
	23	2	4.0
	24	1	2.0
	25	9	18.0
	26	2	4.0
	27	2	4.0
	29	2	4.0
	31	1	2.0
	34	3	6.0
	35	3	6.0
	36	1	2.0
	37	4	8.0
	38	2	4.0
	39	3	6.0
	41	1	2.0
	42	2	4.0
	Total	50	100.0

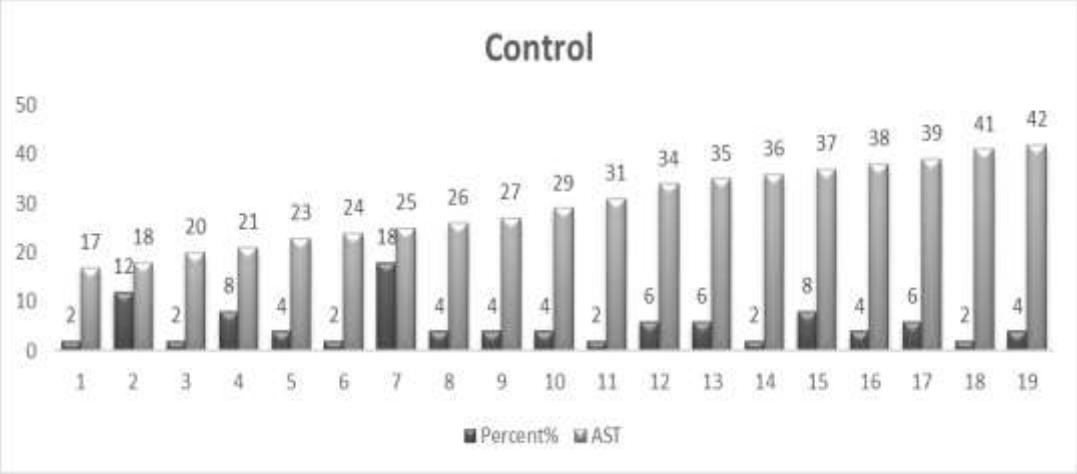

Fig. 8. Percentage of AST results for control group.

The highest percentage of AST was for the cases who their AST was 25 IU/L, the lower percentage of AST was for the cases who their AST was 17, 20, 24, 31, 41 IU/L.

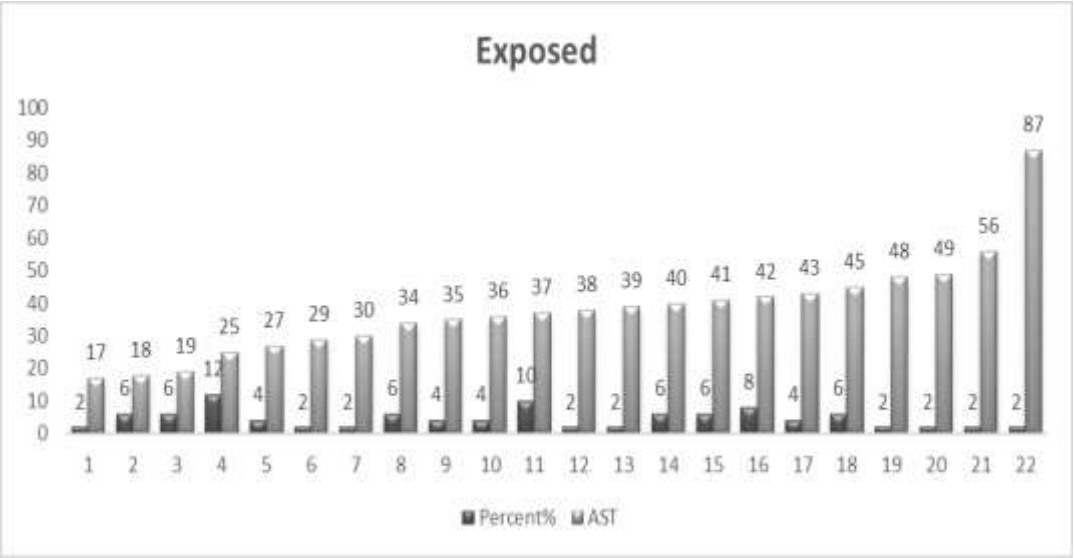

Fig. 9. Percentage of AST results for exposed group.

The highest percentage of AST was for the cases who their AST was 25 IU/L, the lower percentage of AST was for the cases who their AST was 30, 38,45, 48, 56, 87 IU/L.

Table 12. ALT and AST activities (IU/L) of control and exposed groups.

Parameter	Control (N=50)	Exposed (N=50)	P value	Correlation
ALT(IU/L)	**24.16±6.44**	**28.80±7.85**	**<0.05**	**-0.325**
AST(IU/L)	**28.56.2±7.69**	**35.38±12.04**	**<0.05**	**-0.320**

Data represented as mean ± standard deviation (SD), N= number, P < 0.05 significant

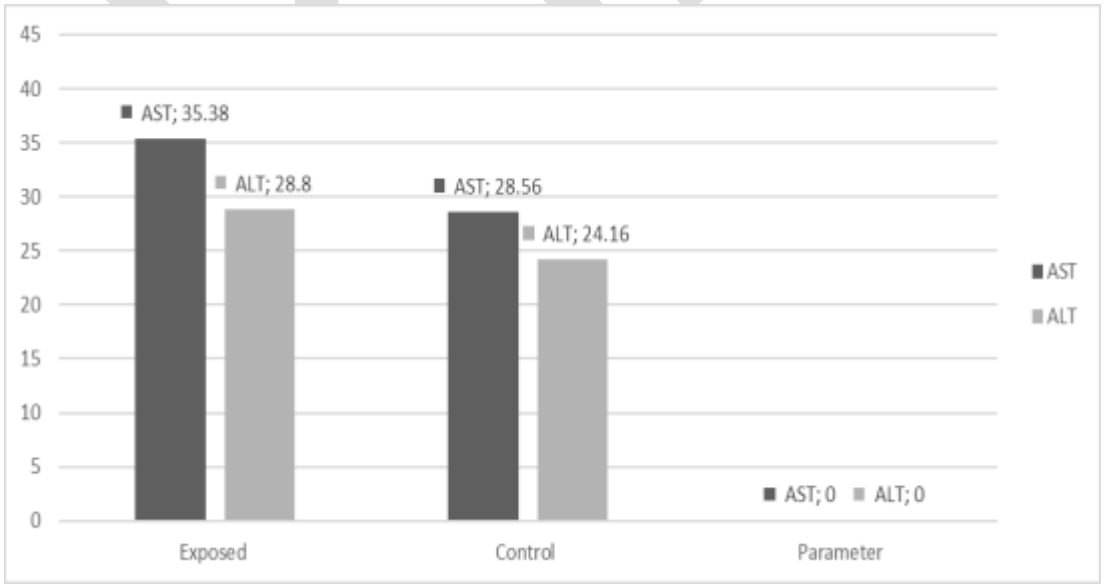

Fig. 10. Comparison between mean ALT and AST in control and exposed groups.

Liver tests performed in this study for cases were usually in the normal range and our findings in fact show the changes of these tests in working population. The clinical importance of these subtle changes of liver function in working populations is not clear and further longitudinal studies are necessary. Considering the results of this study, performing biochemical liver tests is recommended for screening and surveillance of workers exposed to low level VCM.

Our results are in agreement with previous studies that reported deterioration of liver enzymes as a result of plastics exposure among Egyptians **(Henry J et al., 2000; Saad. A et al., 2000; Hsieh H et al., 2003; Ladou J, 2004; Hsiao T et al., 2004; Hassan ZK et al., 2012; Eid JI M et al., 2015; Abd El Dayem SM et al., 2016; Faheem M et al., 2016;).**

In workers who exposed to plastic components showed higher AST and ALT ($P < 0.05$) as compared to control. This shows that exposure to plastic components can aggravate hepatic disorders and further increase liver enzymes. Between these two liver disorders. The normal concentrations in the blood are from 10 to 38 IU/L for AST and from 10 to 40 IU/L for ALT.

DISCUSSION

Regarding ALT and AST as hepatotoxicity biomarkers, the results revealed significant increase in ALT and AST activity of exposed group as compared to the control, also AST in exposed group is higher than ALT which give indication for AST that affected by exposure to plastic more than ALT. The elevation of transaminases resulted from cumulative leakage of both enzymes from ruptured cells due chronic occupational exposure to plastics, consequently accumulation of these enzymes in the blood as reported previously by **(Hsiao T et al., 2004).**

It is important to say that workers at plastic production unit have simultaneous exposure to other materials such as pvc, ethylene gas, but the measured levels of these materials were zero or negligible and they cannot affect liver enzyme levels.

CONCLUSION

It is concluded from these results that plastics exposure adversely affects worker's health status by significantly altering their liver parameters. Further studies are to be needed to identify more risk factors of exposure to plastics considering large population.

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