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ORIGINAL ARTICLES

Studying the impact of exposure to organic solvents on kidney function in occupationally exposed workers

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ABSTRACT

The results of studies on the effect of exposure to organic solvents on kidney in workers occupationally exposed to organic solvents over the last twenty years are contradictory. This is an analytical study conducted on 63 male workers in one of the biggest paint manufacturing factories in Egypt. They exposed to organic solvents during their work and matched in this study with 100 control individual engaged in administrative tasks in organization outside the factory. The controls were not exposed occupationally or recreationally to organic solvents. We studied the effect of occupational exposure to organic solvents on renal function using both conventional renal function tests namely; serum urea, serum creatinine, estimated glomerular filtration rate and two of the early urinary renal biomarkers namely; N acetyl-B-D glucosaminidase and B2 microglobulin. The results of the study revealed statistically significant difference between the Urinary –N-Acetyl glucosaminidase activity (NAG index) (P-value=0.002) in the workers that exposed to organic solvents and their matched controls. The proportion of exposed workers that have abnormal NAG activity (52.2%) is higher than that in their matched controls (24%), which represent more than two fold increase in the activity with high statistically significant difference (p-value=0.002). Results of this study point to a possibility of early renal effects, but not serious to influence the routine kidney function tests at the current levels of occupational exposure to organic solvents.

Key words: Organic solvents – Renal impairment- Early renal biomarkers – NAG - B2M.

Introduction

Chronic kidney (CKD) disease is a major public health problem throughout the world. Adverse outcomes of chronic kidney disease can be prevented through early detection and treatment (Mangione &Dal Canton, 2001). Organic Solvents represent an important group of environmental pollutants to which people are exposed daily in the household settings and workplace. They are present as ingredients in paints, varnishes, lacquers, glues, adhesives, degreasers, cleaners, printing inks, agricultural products and pharmaceuticals (Bale *et al*., 2011). Case reports, case studies and experimental animals' studies demonstrated that solvents are causing a variety of kidney disorders including acute and chronic kidney failure (Price, 2000). Glomerular filtration rate (GFR) is the best overall index of renal function but often impractical in the clinical setting. Consequently, serum creatinine and creatinine clearance are the most widely used methods of assessment of renal injury. Serum creatinine level is considered specific but not very sensitive as its levels do not significantly increase until the GFR is reduced to less than 50% of normal. In addition, its concentration is significantly influenced by several factors including muscle mass, dietary intake, changes in tubular secretion, as well as interference of various drugs and endogenous substances with its assay (Abu-Omar,2005).As renal damage from solvent exposure may remain clinically silent for many years due to the large functional reserve capacity of the kidney, it is necessary to apply sensitive, reliable early indicators (''biomarkers of effect'') to detect early effects and prevent further damage (Voss *et al*.,2005). Specific urinary measurements of low molecular weight molecules of protein such B2 microglobulin is quite sensitive for any tubular injury, but they are not specific for any disease (NKF/KDOQI, 2002). Urinary *N*-acetyl-β-D-glucosaminidase (U-NAG) is a renal tubular enzyme present in normal urine and its activity is elevated in many renal diseases it has emerged as a sensitive marker of early nephrotoxicity. It is the most sensitive and reliable indicator of all urinary enzymes in the early detection of renal impairment (*Skálová*, 2005) as its activity was shown to be increased in the early stages of renal injury, before the onset of abnormalities in the excretory function (price,2000). The results of the studies performed to study the effect of exposure to organic solvents on kidney in workers occupationally exposed to organic solvents over the last twenty years are contradictory. Tubular, glomerular, or no effects were found (Jakubowski, 2005).

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Moreover, some of these studies were criticized for their methodological weaknesses with respect to sample size and inaccuracy in case definition. In addition, few studies tested the kidney function of people with chronic occupational exposure to organic solvents. The aim of this work is to study the effect of occupational exposure to organic solvents on renal function using both conventional methods as well as two of the proved early renal biomarkers.

Aim of the study:

1) Studying the risk of renal affection among individuals exposed to organic solvents as nephrotoxic substance.
2) Identification of the early markers needed to diagnose kidney impairment in the early reversible stages.

Subjects and methods:

This study was carried out in one of the biggest factories manufacturing paints, North Cairo, Egypt.

Subjects:

The study population consists of two groups:
1) Workers group (Exposed): composed of 63 industrial workers currently exposed to the organic solvents during work.
2) Control group (not exposed either occupationally or recreationally) consists of 100 of clerks engaged mainly in administrative tasks in organization away from the factory and haven't been exposed occupationally to organic solvents neither currently nor in the past. They are matched with the exposed group in number, sex and age group (5 year interval).

Exclusion criteria:

All current and ex-smokers as well as all participants having history of diabetes mellitus or hypertension were excluded from the study.

Methods:

The two studied groups were subjected to:
I- An interview to complete a prepared field tested questionnaire form.
II- Full clinical examination: to evaluate the health status of the workers and their matched controls. It included general and abdominal examination with emphasis on the signs of chronic renal disease (puffiness around eyes, lower limb edema, high blood pressure, ect…)
III- Laboratory investigations :
a) Blood samples were collected from both exposed and non exposed individuals by sterile disposable syringes. Each sample was left to clot and centrifuged. The separated serum was used for estimation of routine Kidney function tests namely;
⇒ Serum urea using the enzymatic method for determination of urea concentration in serum (Paton and Crouch, 1977) with reference values 0.15-50mg/dl. Values above 50mg/dl have been considered elevated.
⇒ Creatinine using a colorimetric, alkaline picrate method (Jaff□) (Bartels, 1971) with Reference values: 0.6-1.4mg/dl for males. Values above 1.4mg/dl have been considered elevated.

High performance diagnostic reagent kits had been used for determination of Serum urea and creatinine in this study.
⇒ The glomerular filtration rate (GFR) was calculated individually for each person using *Modification of Diet in Renal Disease (MDRD)* formula that recommended by NKF-K/DOQI clinical practice guidelines. This formula depends on 4 variables(serum creatinine, age, gender and race):

Estimated GFR= 186 X (serum creatinine) $^{-1.154}$ X (age) $^{-0.203}$ X (0.742 if female) X (1.212 if African American). According to National Kidney Foundation-K/DOQI, 2002 guidelines, we considered that GFR <90 mL/ min/1.73m^2 as decreased level.
b) Urine samples in sterile labeled containers were collected in the morning from all individuals then centrifuged to remove insoluble salts and debris. Aliquots were stored at -20°C for the estimation of the early markers of renal damage namely;
- Urinary B2 microglobulin using immunometric enzyme immunoassay for quantitative determination of (B2MG) concentration in urine. A commercial kit (International - Immuno-Diagnostics). 1155 CHESS DR

#121 FOSTER CITY, CA 94404.PHONE: 650-345-9518, FAX: 650-578-1810. EMAIL: intli-d@ix,Netcom.com. Normal reference value is 0-0.3 µg/ml in urine.

- *N*-acetyl-β-D glucosaminidase (NAG) using the colorimetric method (Noto *et al*., 1983) with normal reference values 4.2±1.2mU/mg of urinary creatinine. Because NAG activity is known to vary with age and diuriesis; hence, a NAG index (ratio of NAG activity to urinary creatinine) was calculated to minimize variability. A commercial kits (FAR NAG kit) for colorimetric determination of N-Acetyl-B-D-Glucosaminidase in urine and in serum manufactured by FAR srl via Fermi, 12-37026 Pescantina – VERONA-ITALY. Phone: +390456700870 Fax: +390457157763.website: http://www.fardiag.com. The early markers were determined only for 46 exposed worker and 46 of their matched controls selected randomly due to financial causes.

IV- Data collection tools:

A prepared field tested questionnaire form was completed through personal face to face interview with the study groups individually. The following items were covered:

a) Personal data (name, age, sex, current and past residence, education, marital status, special habits such as smoking and alcohol intake).
b) Detailed occupational history (the current and past job, occupational title, work tasks and the duration of work years, days and hours, the use of personal protective equipment (gloves, goggles, apron, mask) was also considered.
c) Detailed medical history with great emphasis on the symptoms of acute and chronic renal problems (Loin pain, oliguria, and dysurea, frequency of micturation,changes in urine color, ect…) history of diabetes and antidiabetic drugs, hypertension and antihypertensive drugs, renal disease(namely) and long-term use of certain drug especially non steroidal anti inflammatory drugs.
d) Family history of renal disease or renal failure.
e) Clinical examination findings (anthropometric measures, blood pressure, general and abdominal examination).
f) Results of laboratory investigations (routine renal function tests and early renal biomarkers).

Important calculations used in this study:

◊ Mean arterial pressure (MAP): the average blood pressure in single cardiac cycle in an individual was calculated using the following formula:

MAP = [(2 x diastolic) + systolic] divided by 3 (Zheng *et al*., 2008).

MAP from 70 to 110 mmHg is considered normal.

◊ Blood Pressure: The blood pressure was measured by maintaining the arm-cuff position at the heart level during rest in seated position. Two readings were taken (1-2min. interval) and the mean value of the two measurements was used. The blood pressure was measured by auscultation method using mercury sphygmomanometer (JSH, 2009).

V- Environmental monitoring:

Environmental monitoring reports of the work place which is done routinely in the factory were reviewed to detect the level of pollutants in the air. Measurements were done by the Reference Laboratory – Faculty of Science - Ain Shams University; the laboratory is certified by ISO 17025. Measurements were taken during shift time from different production units. Three measurements were taken from each unit and the mean values were calculated. Samples of indoor air were collected in the production units by active sampling on 8 × 110 mm adsorbent tubes containing activated charcoal at a flow rate of 200 mL/min, using an air sampling pump with electronic flow-control. The flow of the pump was calibrated using a mini-Buck optical calibrator. After 4 h the sampling was stopped by placing caps on both ends of the tubes. The tubes were covered in aluminum foil and stored at 4 °C until analysis, *(Scheepers et al., 2010).*

VI- Data manipulation and statistical analysis:

The collected data, the clinical and laboratory results have been computerized and coded using SPSS version 18.0 soft ware (Statistical Package for Social Science) and statistically analyzed. Data were expressed as mean values ± standard deviation (SD). Ranges, frequency of distributions were estimated for quantitative variables. The mean of quantitative variables of the two comparable groups (exposed group and control group) was compared using the Independent-Samples Student's t-test. In case of high standard deviation, we used median test to compare between two groups.The significance of differences between proportions was tested by the Chi-square test ($\chi 2$). Differences were considered significant with P value ≤ 0.05.The correlations between individual variables were tested using Pearson correlation coefficient (*r*) Values ≤ 0.05 were considered statistically significant.

VII- Ethical considerations:

All the individuals included in the study were informed about the procedures regarding clinical and laboratory tests and their results. The required administrative regulations were fulfilled. Informed consent was taken from the participants with assured confidentiality.

Every participant had separate coded file and all files were kept in Private well closed place. The study protocol was approved by the Ethics Committee of The national research center prior to the work. A copy of ethical clearance was provided to every participant and an Arabic version of the consent was explained to them.

Results:

The total number of exposed workers in the factory is 181 workers, of which only 63 workers were eligible to the study. The entire studied exposed group (n=63) are males living in areas around the factory region–Cairo, Egypt. Their mean age is 38.3±10.4 and age range (25-57) years old. Most of them are married (88.4%). The exposed workers that have either basic education or less form (30%).There are 7 working groups in the factory. Laboratory workers represent the higher proportion (28.5%) followed by paint production workers (15.8%).The range of duration of exposure to organic solvents according to their duration of employment was (1-39) years with mean duration (12.1±9.7) years. It was observed that 48.4% of the workers (n=31) are using the Personal Protective Equipment (PPE) regularly while 51.6 %(n=33) always didn't use PPE. The control group is matched with the workers group (t-value=-0.68)(p-value=0.49) in their age with mean age (39.4±9.3). Their age range was (25-58 years). No signs or symptoms of renal impairment have been detected in both studied groups. The control group (n=100) is matched in their mean arterial blood pressure (89.8±9.1) with the workers group (88.5±8.5) (t-value=-0.95) (P-value =0.34)(non tabulated data).

Table 1: Statistical comparison between the renal function tests of workers and control groups.

Renal function test	Workers exposed to organic solvents (n=63)	Control group (n=100)	t- value	p- value
Serum urea (mg/dl)	32.7±9.9	29.8±7.9	2.1	0.037
Serum Creatinine(mg/dl)	0.97±0.37	1±0.2	-0.7	0.45
Glomerular filtration rate (GFR) (ml/min/1.73m)	127±26.5	118.7±37.4	1.4	0.14

(Table 1) shows that the mean serum urea level of the workers (32.7±9.9 mg/dl) is higher than that of their matched controls (29.8±7.9 mg/dl) with statistically significant difference (P-value=0.037).

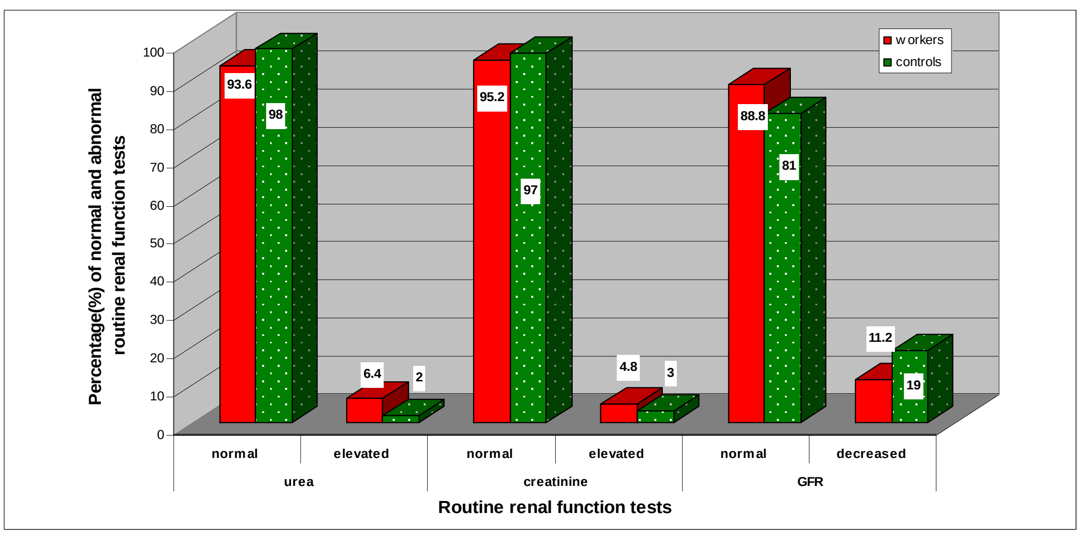

Fig. 1: Bar chart shows percentage of normal &abnormal renal functions in exposed and control groups.

Table 2: Distribution of normal and abnormal renal functions among the studied groups.

Renal function test	Cut off value	Frequency of normal and abnormal values				Pearson Chi-Square test
		Workers (n=63)		Control group (n=100)		
		NO.	%	NO.	%	
Serum urea • Normal • Elevated	50 mg/dl	59 4	93.6 6.4	98 2	98.0 2.0	$\chi2=2$ df=1 p-value=0.1
Serum Creatinine • Normal • Elevated	1.4 mg/dl	60 3	95.2 4.8	97 3	97 3.0	$\chi2=0.3$ df=1 p-value=0.5
Glomerular filtration rate (GFR): • Normal • decreased	90ml/min/1.73m	56 7	88.8 11.2	81 19	81.0 19.0	$\chi2=1.7$ df=1 p-value=0.18
Total		63	100.0	100	100.0	

(Table 2 & Fig.1) reveal that 6.4% of exposed workers have abnormal elevated urea levels which are three times higher than the proportion (2%) of their matched controls but this difference is statistically insignificant (p-value=0.1).

Table 3: Statistical comparison between the renal functions of the exposed workers to organic solvents according to their duration of exposure.

Renal function test	Workers exposed for(<10y) (n=37)	Workers exposed for(≥10y) (n=26)	t-value	p-value
Serum urea	31.9±7.7	34±12.3	-0.8	0.4
Serum Creatinine	0.95±0.36	0.98±0.38	-0.28	0.77
Glomerular filtration rate (GFR)	132±26.9	119.5±24.6	1.9	0.06

(Table 3) the exposed workers are classified according to their duration of exposure into two groups using 10 years intervals (<10y and ≥10y years). This classification reveals insignificant difference in renal function tests among the two groups ($p>0.05$).

Table 4: Correlations between age and duration of exposure with the routine renal functions of the workers.

Renal function test	Age		Duration of exposure	
	r	P-value	r	P-value
Serum urea	-0.1	0.4	-0.01	0.9
Serum Creatinine	-0.26	0.034	-0.18	0.1
Glomerular filtration rate (GFR)	-0.01	0.9	-0.08	0.5

(Table 4) Statistically significant negative correlation is noticed between serum creatinine level and the age of exposed workers. On the other hand, no correlation is observed between renal function tests and duration of exposure.

Table 5: Correlations between the renal functions in the workers.

Renal function	Creatinine		Glomerular filtration rate	
	r	P-value	r	P-value
Serum urea	-0.01	0.93	-0.02	0.87
Serum creatinine*	-	-	-0.65	<0.001

*Correlation is significant at the 0.05 level (2-tailed).

(Table 5) clears the highly statistically significant negative correlation between glomerular filtration rate and serum creatinine (P-value<0.0001).

Table 6: Statistical comparison between the exposed and control groups regarding the activity of urinary renal biomarkers.

Urinary Renal biomarkers	Workers exposed to organic solvents (n=46)	Control group (n=71)	Chi-Square (Median test)	t-value	p- value
Urinary –N-Acetyl glucosaminidase (NAG index)(mu/mg cr) • (Mean ±SD) • Median	7.8±8.1 5.4	3.3±5.6 1.0	— 9.2	3.5 —	0.001 0.002
Urinary B2microglobulin (B2M)(μg/ml) • (Mean ±SD) • Median	0.1±0.09 0.1	0.08±0.1 0.057	— 3.6	1.1 —	0.2 0.058

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Table (6) Clarifies the statistically significant difference (P-value=0.002) in median values as well as mean values of the Urinary –N-Acetyl glucosaminidase (NAG index) in the workers that exposed to organic solvents.

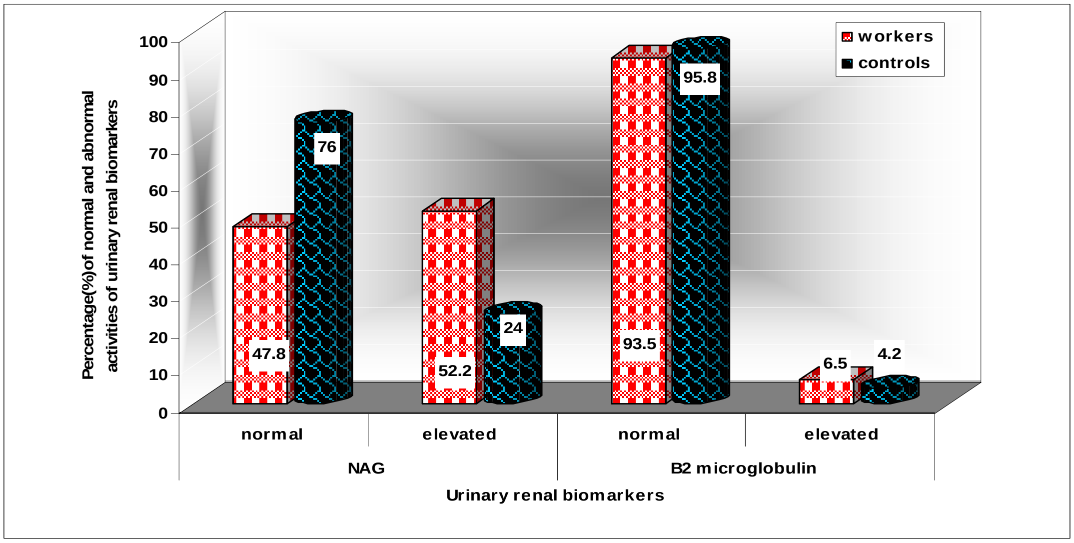

Fig. 2: Bar chart shows distribution of normal &abnormal activities (%) of studied early renal biomarkers in workers and control groups.

Table 7: Distribution of normal and abnormal activities of renal biomarkers among studied groups.

Renal biomarker	Cut off value	Frequency of abnormal values		Pearson Chi-Square test
		Workers group (n=46) NO. %	Control group (n=71) NO. %	
Urinary NAG index* • Normal • Elevated	5.4 mu/mg cr.	22 47.8 24 52.2	54 76.0 17 24.0	X^2=9.4 df=1 p-value=0.002
Urinary B2M • Normal • Elevated	0.3μg/ml	43 93.5 3 6.5	68 95.8 3 4.2	X^2=0.28 df=1 p-value=0.6
Total		46 100.0	71 100.0	

(Fig. 2 & Table 7) show that the proportion of exposed workers that have abnormal NAG activity (52.2%) is higher than that in their matched controls (24%) with high statistically significant difference (p-value=0.002).

Table 8: Statistical comparison between renal biomarkers activities of workers as regards to different durations of exposure.

Studied early renal biomarkers	Workers exposed for(<10y) (n=23)	Workers exposed for(>10y) (n=23)	Chi-Square (Median test)	t-test	p-value
Urinary –N-Acetyl glucosaminidase (NAG index)(mu/mg cr) (Mean ±SD)	9±10	7.7±15.7	–	2.0	0.04
Median	6.8	2.7	2.1	–	0.1
UrinaryB2microglobulin (B2M)(μg/ml): (Mean ±SD)	0.09±0.09	0.1±0.09	–	-1.0	0.28
Median	0.05	0.1	2.1		0.1

Tables (8) reveals statistically insignificant difference in median values of early renal biomarkers among the workers exposed to organic solvents for different durations of exposure where workers are classified into two groups (10 years interval).

Table 9: Correlations between age and duration of exposure with the renal biomarkers in the workers.

Renal biomarker	Age		Duration of exposure	
	r	P-value	r	P-value
Urinary –N-Acetyl glucosaminidase (NAG index)	-0.3	0.046	-0.2	0.17
Urinary B2microglobulin(B2M)	0.03	0.8	0.1	0.3

(Table 9) There is statistically significant negative correlation between the activity of NAG index and the age of workers. No correlation is observed between the two studied early renal biomarkers and the duration of exposure of workers to organic solvents (p-value>0.05).

Table 10: Correlation between the studied renal biomarkers in the workers.

Renal biomarker	UrinaryB2microglobulin (B2M)	
Urinary –N-Acetyl glucosaminidase (NAG index)*	r	P –value
	0.4	0.005

*Correlation is significant at the 0.05 level (2-tailed).

(Table 10) Strong positive correlation between NAG index activity and B2 microglobulin is observed in exposed workers and control groups.

Table 11: Correlations between the studied renal biomarkers and renal function tests in the workers.

Renal biomarker	Urinary –N-Acetyl glucosaminidase (NAG index)		UrinaryB2microglobulin (B2M)	
	r	P-value	r	P-value
Serum urea	-0.02	0.8	0.1	0.2
Serum creatinine	0.06	0.6	0.03	0.8
Glomerular filtration rate	-0.05	0.7	0.07	0.6

(Table 11) No correlations are observed between both studied renal biomarkers (NAG index and B2M) and routine renal function tests (P >0.05).

Discussion:

Exposure to nephrotoxic substances such as organic solvents may cause renal tubular and glomerular dysfunction. This is still a worldwide health issue due to their widespread use as constituents in paints, cleaners, adhesives, inks and many other products commonly found in many workplaces (Ducos *et al*, 2008). Detection of the progressive renal damage using conventional parameters, such as serum creatinine levels (Cr) or creatinine clearance (Ccr) is often misleading (Voskaridou *et al*, 2006). The early development of glomerular hypertrophy enhances creatinine excretion and gives false normal results of both Cr and Ccr. Therefore, the renal dysfunction becomes evident rather late. For that reason, the identification of markers that indicate early renal dysfunction as well as further progression to end stage renal disease is highly desirable (Voskaridou *et al*, 2006). The discovery of several biomarkers in urine and serum in the last decade enables to detect acute renal (tubular) injury and dysfunction early before a decline in GFR and an increase in serum creatinine. These markers may have to meet several requirements to be useful in the clinical settings. They should allow early detection of renal tubular injury reflecting improvement of the kidney injury and be agreeable to rapid and reliable measurement (Bazzi *et al*, 2002).N-acetyl-β-(D)-glucosaminidase (NAG) is a lysosomal enzyme found predominantly in proximal tubules so that increased activity of this enzyme in the urine suggests injury to tubular cells (Liangos *et al,* 2007). The utility of urinary NAG activity as a marker of acute or chronic kidney injury was described more than 30 years ago. In subsequent years, urinary NAG activity was measured to detect mild, subclinical renal tubular damage (Trof *et al*, 2006). B2-Microglobulin (B2M), a low molecular weight protein, is freely filtered in the glomerulus and is totally reabsorbed and degraded in the renal tubules. Thus, it is a sensitive marker of the glomerular filtration capacity of the kidney. B2M serum levels remain low in healthy individuals as their urine contains almost no b2M, while their elevation is associated with the presence of tubular damage (Jacob *et al.,* 2007).

This study was conducted on two groups of individuals; workers group and control group. The Workers group is composed of 63 industrial workers. They are exposed to mixture of aromatic organic solvents (e.g. xylene, toluene, acetone, ethanol and ethyl acetate, ect.), 8 hours daily for 5 days weekly. They have various educational levels ranges from illiteracy up to the higher education. In general words, about two fifth (40.5%) of the factory workers just have the basic education or less (i.e. read and write or illiterate). The range of total duration of exposure of workers was (1-39y) with mean duration (12.1±9.7) years. There are 7 working groups constitute the working manpower in the factory. Paint production and ink production workers represent 28.5% and Laboratory workers represent 28.5% while storekeepers, industrial safety, maintenance workers and others constitute 43%. The two studied groups were significantly different (p-value=0.035) in their educational pattern. About one third (30%) of the exposed workers are highly educated in comparison to (50%) of their matched controls. This finding may explain the high percentage of exposed workers that didn't use personal protective equipment (PPE) (51.6%), in spite of its availability and may reflect low health awareness among them. The possible role of exposure to organic solvents in the development and/or the Progression of chronic glomerulonephritis (GN) is still a controversial scientific issue more than 30 years after the earliest studies by Beirne and Zimmermann,1975 (Kaukiainen *et al.*,2004). The results of our study showed statistically significant difference between the workers that exposed to organic solvents and their matched controls in serum urea levels (P-value=0.037). The mean serum urea level of the workers (32.7±9.9 mg/dl) was higher than that of their matched controls (29.8±7.9 mg/dl). Statistically insignificant differences were observed in mean values of both serum creatinine and glomerular filtration rate in both studied groups (Table 1). Our results revealed statistically insignificant difference between the occupationally exposed workers of the paint factory and their matched unexposed controls in the proportions of renal function tests (p>0.05). 6.4% of exposed workers have abnormal elevated urea levels which are three times higher than the proportion of their matched control (2%).On the other hand, the exposed workers that have abnormal elevated serum creatinine are higher than (4.8%) that in their matched control group (3%) but this difference is also statistically insignificant(Table 2&Fig.1).This result may carry clinical significance in the medical field in general and occupational medicine especially despite of its statistical insignificance which may be attributed to the working in the factory under controlled environmental conditions. The health state of the workers are evaluated annually by occupational health physician of the factory, different investigations are done including kidney function tests. The factory also has industrial safety management department. Environmental monitoring is done routinely in the factory by the Reference Laboratory – Faculty of Science - Ain Shams University; the laboratory is certified by ISO 17025, to detect the levels of pollutants (organic solvents) in the air in different departments. The measured levels of different solvents in working atmosphere show that, all air measurements were below maximum allowable limits according to Egyptian law 1994 and international regulations. To study the effect of duration of exposure to organic solvents among the exposed workers on routine renal function tests, we classified the workers according to their number of working years considering the past exposure years to organic solvents, into 2 groups using 10 years intervals (<10 and,≥10years). This classification revealed insignificant difference in routine renal function tests between the two groups (p>0.05) (Table 3). Moreover, when we studied the correlation between routine renal function tests, age of workers as well as their duration of exposure, statistically significant indirect negative correlation between serum creatinine and the age of workers was observed. No correlation was observed among renal function tests and the duration of exposure to organic solvents (Table 4). These findings may be due to exclusion of older workers from production processes to be work in administrative or observational tasks with little exposure to organic solvents. Another possibility is that the older workers may have the experience to properly use the personal protective equipments as well as they may be keener to protect themselves from hazards of exposure than younger workers. Kaukiainen A *et al*., 2004 found that the serum creatinine level correlated negatively with long term exposure to organic solvents which is in agreement with our finding. In our results, high statistically significant negative correlation between the glomerular filtration rate and serum creatinine (P-value<0.0001) was observed (Table 5).

It is clear that serum creatinine and other routine markers are not very sensitive and are generally only raised when acute renal injury or chronic renal injury is well established(Lock,2010). Biological monitoring of early effects of exposure to organic solvents on kidney can help in identifying individuals susceptible to nephrotoxicity of this group of chemicals (Fathy *et al*., 2009). Measurement of certain enzymes such as N-acetyl-β-(D)-glucosaminidase has been used in both animals and man for many years to provide insight into the onset of renal injury. (Lock, 2010).

In the present study we have studied the excretion of two approved renal biomarkers in detection of early renal impairment according to the literature, low molecular weight protein (LMW) B2 microglobulin and a lysosomal enzyme: urinary N-acetyl B-D- glucosaminidase. Fathy, *et al.,* 2009 Stated that the detection of renal tubular proteins and enzymes may precede glomerular involvement, as several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminurea. The degree of health effect produced by each organic solvent depends on solvent volatility, with generation of significant airborne concentrations of vapor, large surfaces from which evaporation may take place, lack of appropriate enclosure and/or exhaust ventilation systems, and relatively high temperature of the work environment may all contribute to increased uptake by inhalation. The degree of physical exercise required by the actual work performed (increasing the ventilatory volume per minute and thus the amount of solvent vapor absorbed) may be an important determinant of solvent uptake through inhalation, and enhanced levels of physical activity have been associated with uptake rates increased by one to three times their baseline levels (Astrand *et al.,*1972). Bazzi, 2002 who reported an increase in the urinary excretion of N-acetyl-glucosaminidase (NAG) in subjects exposed to substances toxic for renal tubular cells such as organic solvents. This statement is supported by our results where we found statistically significant difference in both mean (P-value=0.001) and median values (P-value=0.002) of the Urinary –N-Acetyl glucosaminidase activity (NAG index) in the workers that exposed to organic solvents and their matched controls with higher mean and median values of the workers than that of their matched controls

(Table 6). This may be due to the dysfunction of tubular epithelial cells induced by increased traffic of proteins in the tubular lumen (Bazzi, 2002). An evidence for the worse effect of exposure to organic solvents on renal performance was achieved in our results when we found that the proportion of exposed workers that have abnormal NAG activity (52.2%) is higher than that in their matched controls (24%) and represent about two fold increase in the activity with high statistically significant difference (p-value=0.002) (Table 7&Fig.2). Brogren *et al,* 1986 also coincided with our results as they reported an increase in NAG activity in 10% of workers exposed to organic solvents than their matched controls. Several occupational cross-sectional studies have shown that workers who were exposed to various types of solvents had higher levels of low molecular weight proteinuria, a marker of tubular injury, as compared with non exposed workers (Hotz,1994) in contrary to our results where a statistically insignificant difference in proportions of workers that have abnormal B2M activity and their matched controls ($p > 0.05$) which may be explained by the sensitivity of each renal biomarker to detect specific renal impairment. B2M is more sensitive in detecting proximal tubular injury, as compared with that of NAG (Price, 1992) while NAG could be elevated in both glomerular diseases as well as in tubular injury. Hashimoto, 1995, reported that high BP is one of the causes of elevated urinary NAG activity in the general population. In this study, to eliminate the role of high blood pressure in increasing NAG activity, we excluded any individual has history of hypertension or history of taking antihypertensive drugs. Moreover, we found that, there is matching between the exposed workers and their matched controls (p-value=0.34) (Table 1). According to our results, there is statistically insignificant difference in median values of NAG index activity among exposed workers with various durations of exposure(Table 8) while there is statistically significant negative correlation between NAG activity and age of workers(Table 9).This finding may confirms our former explanation that the older workers may be engaged in tasks less exposed to organic solvents than younger one in the same workplace due to their seniority or due to their health state. Another explanation for our finding is that the absenteeism may be higher in older workers that may consider as chance for the NAG activity to return back to their normal levels. This explanation is in agreement with Price RG, 1992 who reported that the NAG activity remains high during active disease or a sustained toxic exposure but falls to normal levels on recovery or removal of the toxin. The correlation between the two renal biomarkers was studied revealing statistically significant positive correlation between the NAG and B2M activities in the studied workers exposed to organic solvents (r=0.4) (P-value=0.005) (Table 10). Our finding is coinciding with Bazzi, 2002 who found significant positive correlation between NAG excretion and B2M, the protein that reflects tubular impairment. When we studied the correlation between renal biomarkers activities and routine renal function tests, no correlations were noticed among them (Table 11). The increase in N-acetyl glucosaminidase (NAG), the enzyme that produced by renal proximal tubular cells and has been widely used as a marker, indicates a degree of renal tubular damage (Fujita *et al*., 2002). It showed higher activity in our study in the occupationally exposed workers to organic solvents despite their routine renal functions were still within normal range or mildly affected (Bazzi, 2002). These findings in addition to absence of symptoms and signs of renal impairment in both studied groups are in agreement with Mangelsdorf, 2009 statement "Under conditions of chronic solvent exposure, renal damage may remain clinically silent for many years owing to the large functional reserve capacity of the kidney. During this time, alterations may gradually progress through a cascade of events, from early biological effects that may be of no clinical significance through initially reversible functional and/or structural alterations to focal damage, and finally to manifest, clinically detectable disease". Krusell *et al*, 1985 studied the effect of chronic occupational exposure to organic solvents (average 15y) in 43 printing workers on renal function through determination of urinary B2M and albumin excretion and they concluded that moderate chronic exposure to organic solvents among active printing industry workers does not adversely affect the kidneys. These statements may explain our findings where the B2M showed insignificant increase in its activity in contrary with NAG activity indicating more sensitivity of NAG rather than B2M in detecting the renal impairment among workers exposed to organic solvents. The results of this study points to a possibility of early renal effects, but not serious to influence the routine kidney function tests at the current levels of occupational exposure to organic solvents.

Conclusion:

The urinary N-acetyl-beta -D-glucosaminidase was found to be significantly higher in occupationally exposed workers to organic solvents than their matched controls, in one of the environmentally controlled paint manufacturing factories. This study supports the evidence of the potential role of exposure to mixture of organic solvents in impairing the renal functions.

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