Basrah University

College of Pharmacy

White Blood Cells Activity Assays of Individuals in Industrial and Urban Areas

بحث التخرج للمرحلة الخامسة

لسنة 2015 - 2016

اعداد الطالبات:

1- نغم نعيم فزع

2- إبتهال باقر موسى

باشراف :

الدكتور- عامر خزعل

الدكتورة – زينب نجم

1. Introduction

Employment in industrial areas away from urban territories causes exposure to many toxic chemical and physical agents, along with hard climate changes and environmental hazards such as air pollutants and factory harmful bi-product emissions. The site workers and office employee in their rooms are usually exposed to multiple agents while performing their tasks, and may suffer from potential adverse health effects such as respiratory, hematological, hepatic, and renal problems. (Wald and Jones,1987: Harrison,1992: Teitlebaum,1986).The purpose of this study is to assess the risk by surveying some clinical investigations and health parameters along with conducting hematological and liver function tests in The State Southern Company of Fertilizer in Basra Governorate, city of Iraq.

The liver function tests and thehematological data from both urban and industrial employees were encoded and analyzed statistically in Microsoft Excel program using student's *t* test and analysis of variance (ANOVA) to differentiate between the working sections. Arise in plasma aminotransferase activities is a sensitive indicator of damage to cytoplasm and/ or mitochondrial membranes. Plasma enzyme activities rise when the membranes of only very few cells are damaged. Liver cells contain more aspartate amino- transferase (AST)than alanine aminotransferase (ALT), but ALT is confined to the cytoplasm, in which its concentration is higher than that of AST. Raised plasma levels of both enzymes are indicative of hepatocyte damage. In inflammatory or infective conditions such as hepatitis, leakage of cytoplasmic contents causes a relatively greater increase in plasma ALT than AST activities. On the other hand, in infiltrative disorders in which there is damage to both mitochondrial and cytoplasmic membranes, there is a proportionally greater increase in plasma AST than ALT activity. A plasma AST:ALT ratio of >2 is suggestive of intoxicative liver disease and a ratio <1 suggests chronic hepatitis or hepatic steatosis (Luo et. Al., 1998).

Liver function abnormality was defined as serum ALT>36 U/L, and serum AST>34 U/L. (Azhar et.al.,2015)

2. Materials and Methods

2.1. Clinical investigation

This study is part of clinical investigation conducted on 30 workers from the state company of fertilizers and employments in Basra university campus. Information was gathered concerning occupational history of working periods, lifestyle such as smoking, health status of chronic illnesses like hypertension and diabetes. Physical examination of arterial blood pressure and heart pulse rate were taken for each individual along with body temperature and measuring oxygen saturation (SpO2) using Rossmax-fingertip Pulse Oximeter.(Fig.1)

PDF-XCHANGE
Click to buy NOW!
www.tracker-software.com

Fig. (1) Rossmax- Pulse Oximeter

2.2. Hematological study

The EDTA anti-coagulated blood samples were kept in a cool box until arrival to university campus for blood CBP assays, then part of blood was transferred to gel tubes and centrifuged for serum collection. All study subjects had complete blood picture (CBP) test for hematology parameters concerning white blood cells using Automated Hematology Analyzer COUNT-60, U.S.A (Fig.2). The serum collected using gel test tubes, was used for assessing liver enzymes function: Alanine Aminotransferase (ALT) and aspartate aminotransferase (AST).

PDF-XCHANGE
Click to buy NOW!
www.tracker-software.com

Fig.(2) Genex Laboratories Hematology Analyzer

2.3. Liver Function Tests

ALT and AST enzyme kits used were RANDOX for the colorimetric determination of the serum alanine aminotransferase and serum aspartate aminotransferase respectively, according to Reitman and Frankel.[11]The procedure involved measurement of optical density (OD) against a reagent blank using spectrophotometer at wavelength 546 nm.,and reconstruction of a calibration curve using Pyruvate standardas in the following table (1)

Table (1) Preparation of standard curve solutions

Tube No.	Pyruvate Standard (ml)	Redistilled Water (ml)	Buffer Solution (ml)
1	0.00	0.2	1.00
2	0.05	0.2	0.95
3	0.100	0.2	0.90
4	0.15	0.2	0.85
5	0.20	0.2	0.80
6	0.25	0.2	0.75
7	0.30	0.2	0.70
8	0.35	0.2	0.65
9	0.40	0.2	0.60
10	0.45	0.2	0.55

The procedure involves pipettingreagents into test tubes as above thenmix and pipette into each tube 1.0 ml of reagent 2,4-DNP solution, mix and incubate for 20 min at 20^{o}C. After that add 10 ml of sodium hydroxide solution (0.4 mol/l) to each tube.

Mix and read absorbance against blank (tube no. 1) after 5 minutes. The absorbance of the increasing amounts of pyruvate (0.05- 0.45 ml pyruvate standard) correspond to the following transaminase activities in U/L. as in the table (2) below:

Table (2) The amount of pyruvate corresponding to transaminase activity

Tube No.	ALT U/L	AST U/L
2	9	6
3	18	11
4	27	16
5	37	20
6	46	25
7	56	31
8	67	37
9	77	44
10	87	52

The calibration curve is obtained by plotting the measured absorbance against the transaminase activities in U/L. (Ordinate = absorbance, Abscissa = activity in U/L)

For serum test pipette into test tubes as in table (3)

Table (3) Preparation for serum measurements against Blank

	Reagent Blank	Sample
Sample	---	0.1 ml
Buffer	0.5 ml	0.5 ml
Distilled Water	0.1 ml	---
Mix incubate 30 min at 37°C		
Reagent 2,4-DNP solution	0.5 ml	0.5 m
Mix, allow to stand for exactly 20 min. at 25°C		
Sodium Hydroxide	5.0 ml	5.0 ml

Mix, then read the absorbance of sample against the reagent blank after 5 min.

Hematology reports were classified according to urban and industrial groups and comparison between averages were done statistically using student's *t* test.

3. Results

3.1 Clinical investigation

Health parameters means ± SD error and clinical investigations for urban workers are presented in figures (3), (4).

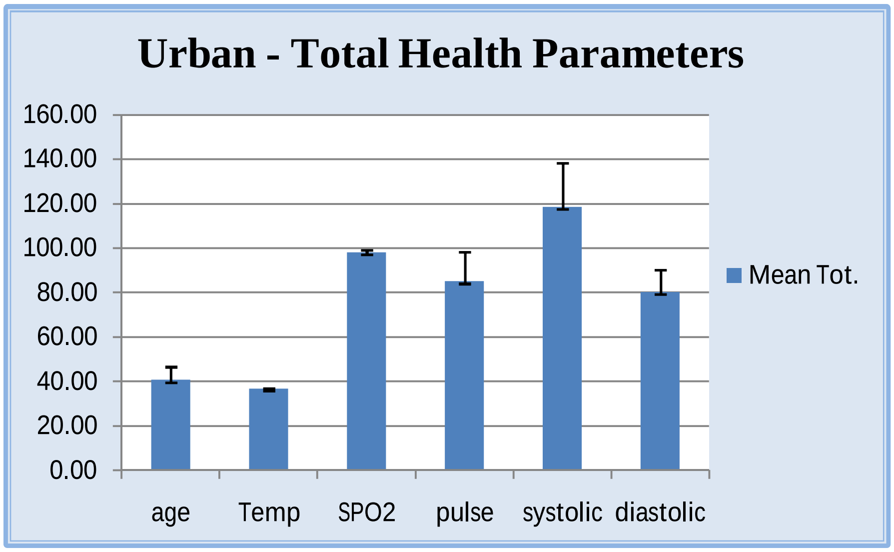

Fig. (3) Urban workers health parameters presented as Mean ± SD

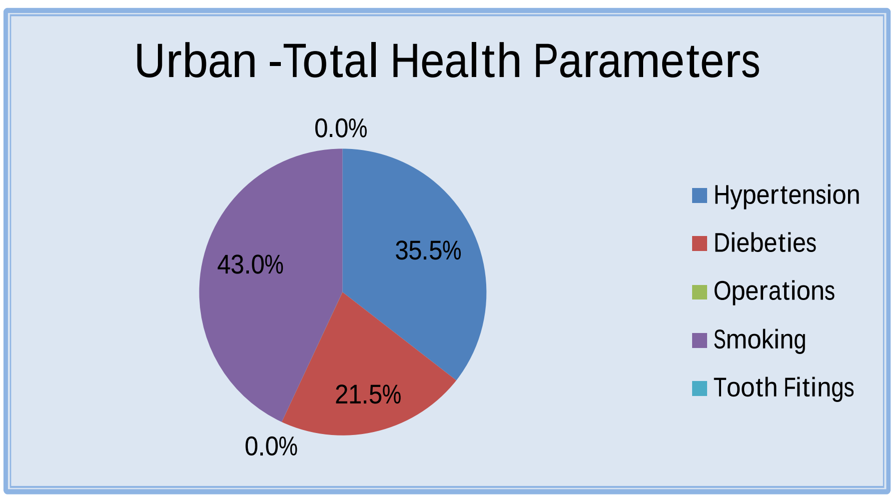

Fig. (4) Urban workers clinical investigations in percentages

Health parameters means ± SD error and clinical investigations for industrial workers are presented in figures (5), (6).

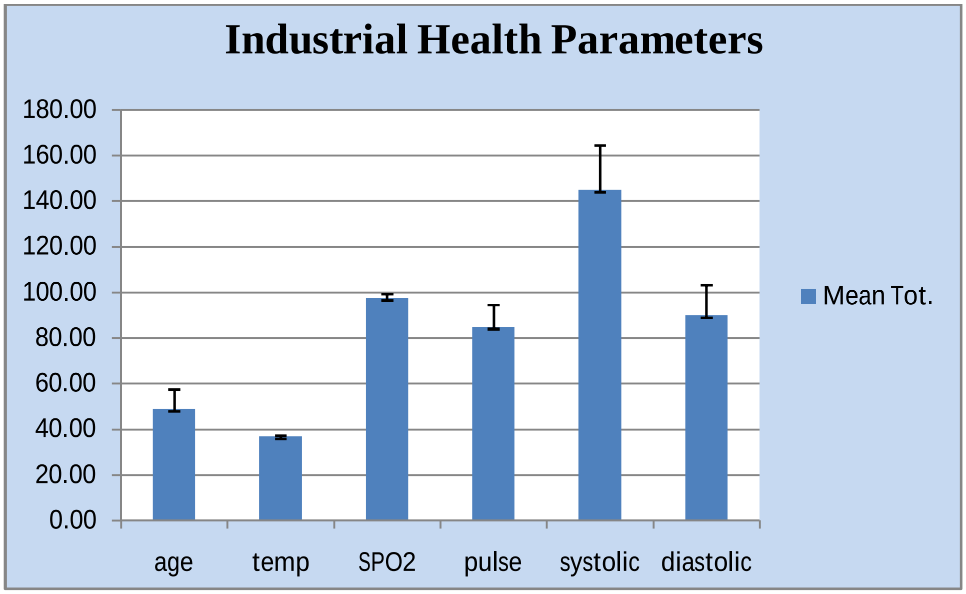

Fig. (5) Industrial workers health parameters presented as Mean ± SD

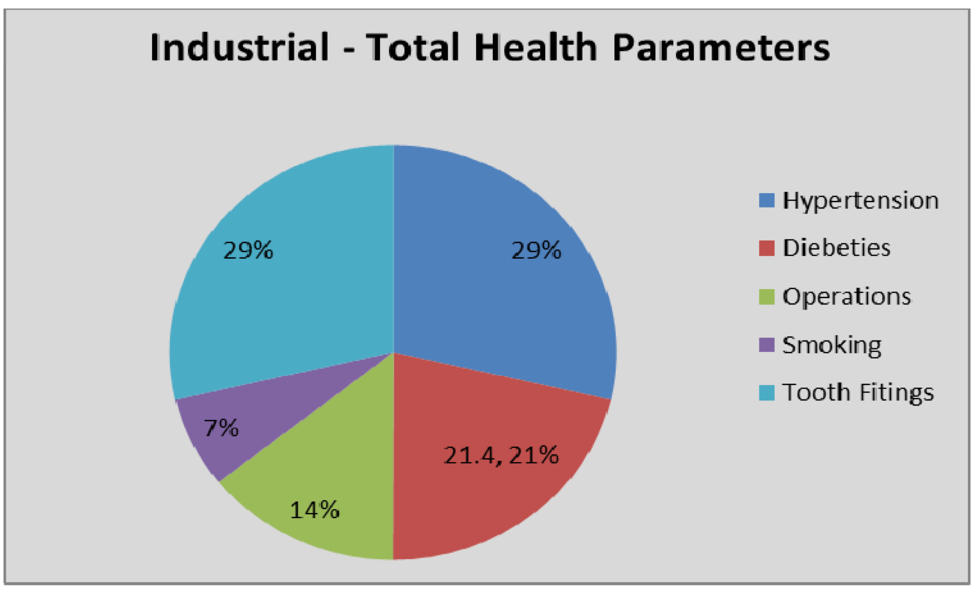

Fig. (6) Industrial workers clinical investigations in percentages

The study showed higher incidence of chronic diseases like hypertension and diabetes between urban workers than in industrial workers,

PDF-XCHANGE Click to buy NOW! www.tracker-software.com

while more industrial worker undertaken surgical operations and had dentistry tooth fittings and are less in smoking.

Urban and Industrial workers Health parameters calculated for Means ±SD are presented in table (4).

Table (4) Urban and Industrial workers Health parameters Means ±SD

n=15	age	Temp	SPO2	pulse	systolic	diastolic
UrbanMean	40.73	36.82	98.27	85.12	118.54	80.31
SD±	5.94	0.28	1.00	13.15	19.72	9.90
Industrial Mean	48.93	37.00	97.57	85.07	145.00	89.93
SD±	8.53	0.46	1.91	9.42	19.48	13.49
P=	0.016	0.260	0.151	0.344	0.001	0.106
(P<0.05)*	*	-	-	-	**	-
(P<0.01)**						

From table (4) there is a significant difference in the age of workers, where industry employees being long exposed to toxic chemical and physical agents, which caused further highly significant increase (P<0.001) in systolic blood pressure. No significant difference was observed in the rest of health parameters investigated.

3.2. Hematological study

Industrial and urban WBCs distribution are presented in figures (7) and (8)

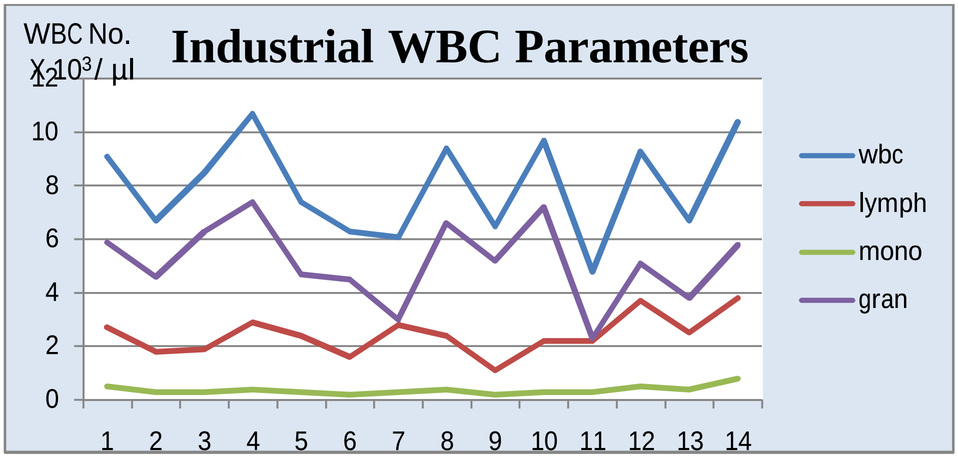

Fig. (7) Industrial workers WBCs parameters distribution

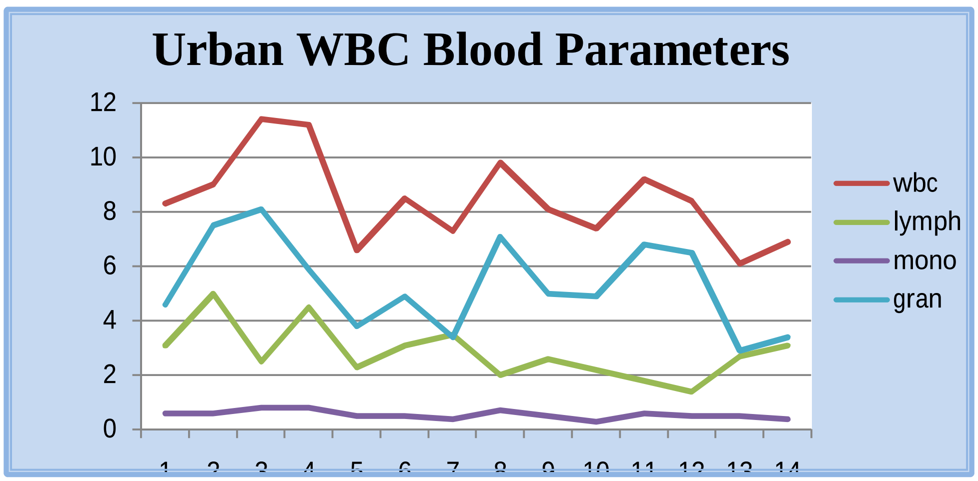

Fig. (8) Urban workers WBCs parameters distribution

This study revealed that both workers had uniformdecreased monocytes levels, while total WBCs and granulocytes follow similar zigzag patterns and were un-uniformly distributed. Lymphocytes in both urban and industrial workers were evenly distributed throughout workers.

Urban and Industrial workers WBCs parameters calculated for Means ±SD are presented in figure (9)

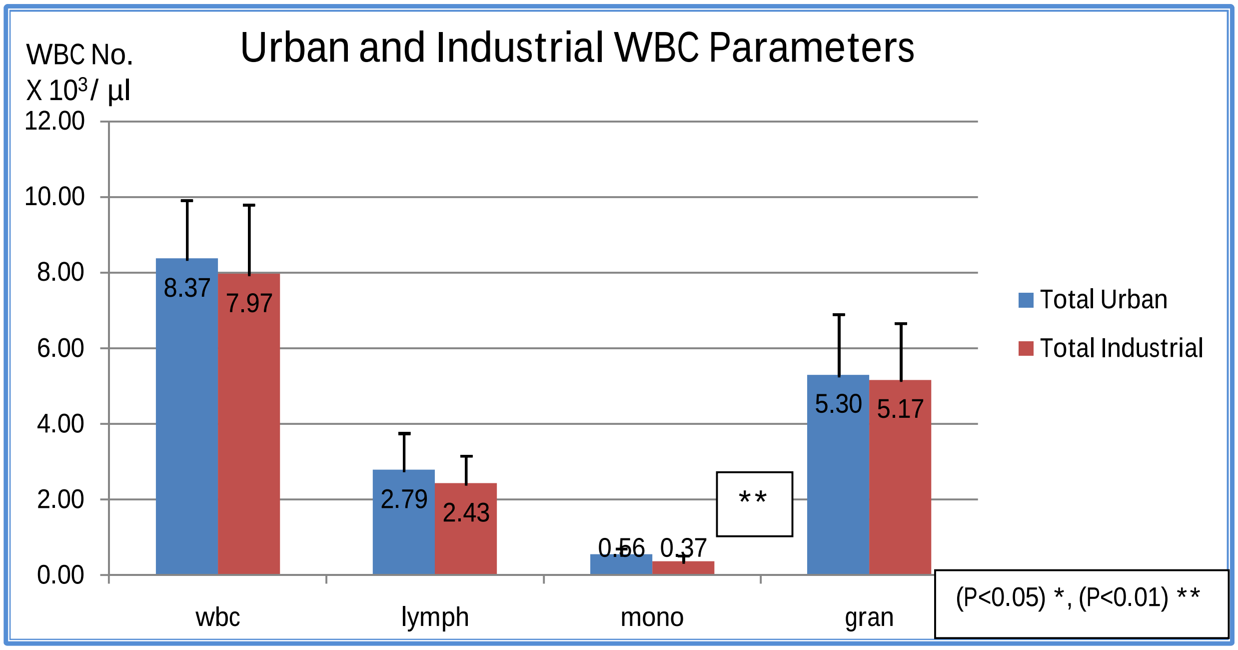

Fig (9) Urban and Industrial workers WBCs parameters Means ±SD

Statistical analysis showed that a highly significant decrease (P= 0.005) is present in industrial monocytes number than urban workers. A general trend of decrease which is present in all other WBCs parameters of industrial workers but with no significance (P> 0.05).

3.3. Liver Function Tests

The absorbance valuesat 546 nm of the increasing amounts of pyruvate corresponding to the alanine transaminase activities in U/L are shown in Table. (5)

PDF-XCHANGE Click to buy NOW! www.tracker-software.com

Table (5) Optical densities according to transaminase activity levels

ALT U/L	Optical Density (OD)
9	0.001
18	0.087
27	0.135
37	0.2
46	o.231
56	0.306
67	0.321
77	0.35
87	0.395

The reconstructed calibration curve is shown as infigure (10)

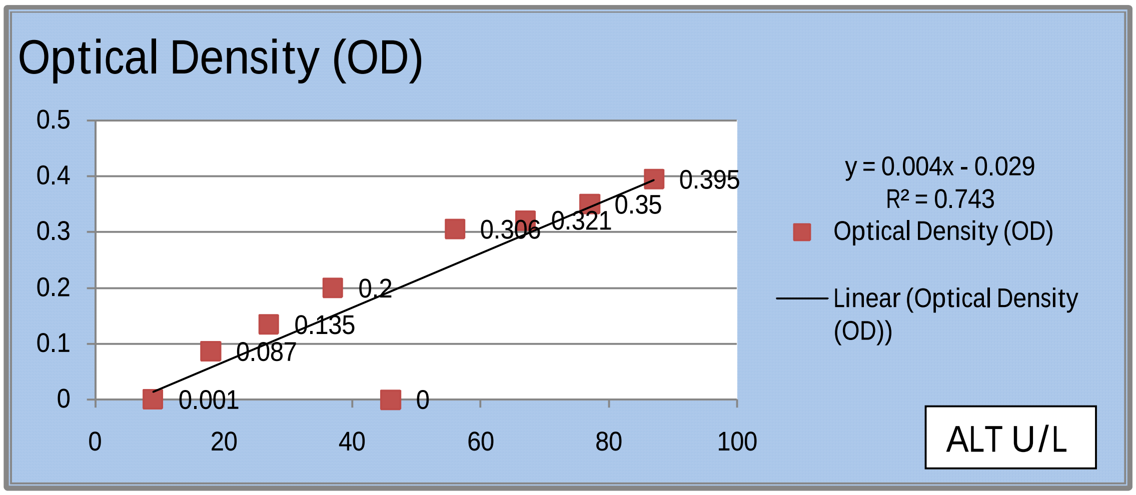

Fig.(10) ALT Enzyme level in Accordance to Absorbance values

The absorbance values at 546 nm of the increasing amounts of pyruvate corresponding to the aspartate transaminase activities in U/L are shown in Table (6)

PDF-XCHANGE
Click to buy NOW!
www.tracker-software.com

Table (6) Optical densities according to transaminase activity levels

AST U/L	Optical Density (OD)
6	0.001
11	0.001
16	0.016
20	0.027
25	0.044
31	0.057
37	0.073
44	0.084
52	0.086

The reconstructed calibration curve is shown as in figure (11)

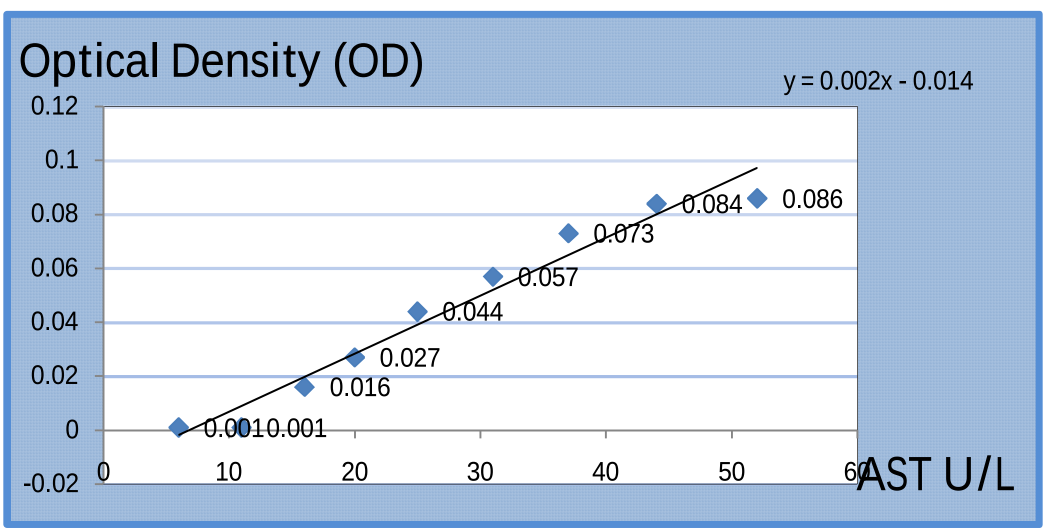

Fig. (11) AST Enzyme level in Accordance to Absorbance values

PDF-XCHANGE Click to buy NOW! www.tracker-software.com

Industrial employees ALT enzyme levels according to the standard curve fittings are shown in table (7)

Table (7) Industrial employees ALT enzyme levels obtained from standard curve

No.	Blood Sample O.D.	Reagent Blank O.D.	Calculated O.D.	ALT (U/L)
1	0.461	0.359	0.102	27
2	0.463	0.359	0.104	27
3	0.461	0.359	0.102	27
4	0.445	0.359	0.086	24
5	0.570	0.359	0.211	49
6	0.556	0.359	0.196	46
7	0.585	0.360	0.225	52
8	0.484	0.360	0.123	31
9	0.433	0.361	0.076	22
10	0.393	0.357	0.034	13
11	0.525	0.358	0.166	40
12	0.515	0.359	0.155	38
13	0.422	0.360	0.062	19
14	0.584	0.360	0.225	52

Industrial employees ALT enzyme levels according to the standard curve fittings are shown in Table (8)

PDF-XCHANGE Click to buy NOW! www.tracker-software.com

Table (8) Industrial employees AST enzyme levels obtained from standard curve

No.	Blood Sample O.D.	Reagent Blank O.D.	Calculated O.D.	AST U/L
1	0.290	0.287	0.003	8
2	0.284	0.289	0.000	7
3	0.301	0.289	0.012	12
4	0.315	0.290	0.025	18
5	0.311	0.290	0.021	16
6	0.314	0.291	0.023	17
7	0.341	0.291	0.050	29
8	0.292	0.291	0.001	7
9	0.315	0.291	0.024	18
10	0.306	0.292	0.014	13
11	0.309	0.292	0.017	14
12	0.308	0.292	0.016	14
13	0.294	0.293	0.001	7
14	0.320	0.293	0.027	19

Liver function tests showed abnormality in 42% of industrial workers (ALT> 36 U/L), while 92.8% were suggestive for chronic hepatitis or steatosis due to ratio less than one of AST: ALT levels.

Urban and industrial employees ALT and AST enzyme levels according to the standard curve fittings were tabulated and calculated for mean and standard error and presented in table (9)

PDF-XCHANGE Click to buy NOW! www.tracker-software.com

Table (9) Urban and Industrial employees ALT, AST enzyme levels Means and ±SD

Urban	**n=15**	**ALT (U/L)**	**AST (U/L)**
	Mean	34.14	37.27
	±SD	7.63	4.58
Industrial	**n=15**	**ALT (U/L)**	**AST (U/L)**
	Mean	33.36	14.21
	±SD	12.8	6.09
(P<0.05)* (P<0.001)***		Not Significant (N.S.)	***

4. Discussion:

This study explained why industrial workerswill generally be employees with a notable risk ofhigher exposures than urbans. The tasks of male processors, maintenanceworkers, and equipment engineers may put them at risk ofintermittent long term peak exposure to toxic gases like ammonia (principal pollutant in fertilizer plant site) as site workers so they will further suffer hematologicaltoxicity, same results obtained byCullen et al. (1983) and Weeks et al. (1991).

Industrial worker health parameters showed decreased mean SPO^2 , pulse rate with high body temperature due to inhalation of ammonia i gas that is highly irritating, colorless andvery soluble. Previous work of Close et al.(1980) showed that It is absorbed in the superior part of the breathingpath where it inflicts pathological and patho-histologicalchanges .

The observed fluctuation (decrease) of respiratory and pulse rate may be related to ammonia-induced change in blood pH (Olanrevaju et al., 2008). Respiratory compensation has been reported to correlate with change in pH (Roller, 1967). The pH of the blood is maintained within a very narrow range during exposure to ammonia and fluctuates along with partial pressure of CO2, O2 (Olanrevaju et al., 2008).

Mohammed et al. (2015) reported that chronic exposure of workers to petroleumfumes like benzene (an aromatic hydrocarbon that is anatural component of crude oil and natural gas) present hugely in the plant atmosphere, is toxic to the blood and blood-formingorgans leading to adverse effects on human hematopoietic system like bonemarrow depression and resultant pancytopenia. Same results in this study obtained where total WBCs, lymphocytes, and granulocytes means of industrial workers decreased, with extremely significant decrease ofmonocytes ($P<0.01$).

Jorunn*et al.*(2008) reported that chronic hematotoxic effects ofbenzene exposure, including reduce lymphocyte, neutrophil and platelet countsin peripheral blood, have been detected atoccupational exposure below a level that hadpreviously been considered not to cause anyhealth effects but whether these abnormalitiesrepresent bone marrow damage and/or initialevents in the development of a trueneoplastic disease is not known

Another study done in Nigeria on fuelattendants showed similar results, with aglobal reduction in the mean values of totalleucocyte count, red blood cell count,Packed Cell Volume and other red bloodcell indices in exposed workers (Okoro*etal.*, 2006).

This study results also went parallel with Luo et.al.(2002) findings where there was highly significant lowermean WBC counts in male industrialworkers (Mean=5870/mm3, SD=1190) versusurban workers (Mean=7350 /mm3, SD=1660), $p=0.003$). The industrial job categoriesincluded cleaning up chemical spills and leaks, cleaningequipment, mixing the chemical, changing solid sources andpump oil, doing preventive maintenance on a machine, doingan emergency response for a machine, and changing or fillingcontainers for chemicals on machines.Male engineers usuallywore cotton masks and cartridge or air masks during theoperation. Lack of adequate respiratory protection may allow workers to be exposed to hematological toxins. One previousreport indicated that engineers might be exposed to high concentrationof toxic chemicals during preventive maintenance. (Strang et al., 1989)

This study correspond with Luo et al. (2002) conclusions were the results of subchronic effects show that thetasks of industrial workers may put them at risk of developinglong term haematological and hepatic abnormalities.

This study showed an extremely significant decrease in liver function tests in industrial workers (Mean=37.2,SD= ± 4.58) than Urban workers (Mean= 14.2, SD= ± 6.09) which coincides with the results of Azhar et. Al. (2015) due to chronic hepatitis due to hepatocytes intoxication with air pollutants and harmful factory by-products.

5. Conclusion:

This study suggests that decreased WBCs (leukopenia) and moderate problems concerning health parameters, along with markers of liver dysfunction is a potential health defect in industrial workersof the fertilizer industry. The tasks of the processing, maintenance, and site equipment engineersput them at risk for recurrent long term exposure to ammonia gases, toxic agents, and other environmentalhazards. The findings of this clinical surveillance are significant;however, a further investigation of the aetiological factors and subsequent pathogenicity is necessary.

References:

Ali, A., Sahb, A. 2011. Hematologicalassessment of gasoline exposureamong petrol filling workers inBaghdad.*J. Fac. Med. Baghdad*,53(4).

Azhar S. Muslim, Raghad H. AL-azzawi,ZainabS.kalief. Prevalence of Hepatitis E Virus in AL-Muthanna Province (Iraq) Patients. (2015)*Iraqi Journal of Science Vol 56, No.2A, pp:965-971*

Bor-Kucukatay, M., R. B. Wenby, H. J. Meiselman and O. K. Baskurt. 2003. Effects of nitric oxide on red blood cell deformability.*American Journal of Physiology*, **284** (5): H1577-1584.

Close, L. G., F. I. Catlin and A. M. Cohn. 1980. Acute and chronic effects of ammonia burns of the respiratory tract. *Arch. Otolaryngol*., 106 (3): 151-158.

Cone JE. Health hazards of solvents. In: Ladou J, ed. The microelectronics industry. State of the art reviews. Philadelphia: Hanley and Belfus, 1986:69–87.

Cullen MR, Rado T, Waldron JA, et al. Bone marrow injury inlithographers exposed to glycol ethers and organic solvents used inmulticolor offset and ultraviolet curing printing processes. Arch EnvironHealth 1983;38:347–54.

Dyavolova, M., I. Yanchev, D. Gudev and P. Moneva, 2013. Effect of high ammonia level on stressinduced hematological changes in rabbits: preventive effect of pyridoxine. *Bulg. J. Agric. Sci*., 19: 828-834.

Gautam, A.K., Chowdhury, A.R. 1987. Effect of lead on erythropoietic system of intact and splenectomizedrats.*Indian J. Physiol. Pharmacol.*,31(2): 117 124.

Harrison M. Semiconductor manufacturing hazards. In: Sullivan JB, Krieger GR, eds. Hazardous materials toxicology: clinical principles of environmental health. Baltimore: Williams and Wilkins; 1992:472–504.

PDF-XCHANGE Click to buy NOW! www.tracker-software.com

Jorunn, K., Trond, R., Tore, G.B., *et al.*, 2008.Effects of benzene on humanhematopoiesis.*Open Hematol. J.*, 2:87 102.

Lewis DR. Dopant materials used in the microelectronics industry. In: Ladou J, ed. The microelectronics industry. State of the art reviews. Philadelphia: Hanley and belfus, Inc; 1986:35–47.

Luo J-C J, Hsieh L L, Chang M J W, and Hsu K-H. Decreased white blood cell counts in semiconductor manufacturing workers in Taiwan. Occup Environ Med 2002;59:44–49 doi: 10.1136/oem.59.1.44

Luo J-C J, Hsu K-H, Hsieh L-L, et al. Lung function and general illnesssymptoms in a semiconductor manufacturing facility. J Occup EnvironMed 1998;40:895–900.

Mohammed Aleemuddin, M.GirishBabu, M.L.Manjunath and ShireenSwalihaQuadri, 2015.Effect of chronic inhalation of petroleum products on hematologicalparameters. Int. J. Curr. Res. Aca. Rev. Vol. 3 (4): 196-201

Monfort, P, E. Kosenko, S. Erceg, J. J. Canales and V. Feligo, 2002. Molecular mechanism of acute ammonia toxicity: role ofNMDA receptors. *Neurochem. Int*., 41 (2-3): 95-102.

Okoro, A.M., Ani, E.J., Ibu, J.O.,Akpohomeh, B.A. 2006. Effect ofpetroleum products inhalation onsome hematological indices of fuelattendants in Calabar metropolis,Nigeria.*Niger. J. Physiol. Sci.*, 21(1-2): 71 75.

Olanrevaju, H. A., J. P. Thaxton, W. A. Dozier III, J. Purswell, S. D. Collier and S. **L.** Branyon, 2008. Interactive effects of ammonia and light intensity on hematochemical variables inbroiler chickens.*Poult. Sci*., **87** (7): 1407-1414.

Pandey, K. B. and S. I. Rizvi, 2011.Biomarkers of oxidative stress in red blood cells.*Biomed Pap Med FacUnivPalackyOlomoncCzech Repub*., 155 (2): 131-136.

PDF-XCHANGE Click to buy NOW! www.tracker-software.com

Roller, M. H., 1967.The effect of acute ammonia toxicity on certain blood parameters in cattle.*Dissertation abstracts*: B 1966,27:1603B-1604B, Record number 1967, 1407707.

Strang CR, Levine SP, Herget WF. A preliminary evaluation of the Fourier transform infrared spectrometer as a quantitative air monitor for semiconductor manufaturingprocess emissions. AmIndHygAssoc J 1989: 50:2:70–7.

Subash, S. and P. Subramanian, 2008.Effect of morin on the level of liver markers and redox status in experimental chronic hyperammonaemic rats.*Singapore Medical Journal*, 49 (8): 650-655.

Teitlebaum DT. Photoactive chemicals used in photoresist systems. In: Ladou J, ed. The Microelectronics Industry. State of The Art Reviews. Philadelphia: Hanley and Belfus, 1986:59–68.

Wald PH, Becker CE. Toxic gases used in the microelectronics industry. In: Ladou J, ed. The microelectronics industry. State of the art reviews. Philadelphia: Hanley and Belfus, 1986:105–17.

Wald PH, Jones JR. Semiconductor manufacturing: an introduction to processes and hazards. Am J Ind Med 1987;11:203–21.

Weeks JL, Levy BS, Wagner GR, eds. Aplastic anemia.: Preventingoccupational diseases and injury. Washington, DC: American Public Health Association, 1991:1059.