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Research Paper

Hematological and immunological effects of gallium, indium, and arsenic in light emitting diodes manufacturing workers

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Abstract

Purpose: To determine whether hematological and immunological changes occur in manufacturing workers in association with gallium, indium, and arsenic biomarkers in light-emitting diodes.
Methods: 91exposed light-emitting diodes, workers and 24 referents were monitored for whole blood and urine levels of gallium, indium, and arsenic. Venous blood was also collected for routine and immunological analyses.
Results: The mean levels of blood in, urine Ga, urine as, and mean corpuscular hemoglobin concentration (MCHC) in the exposed workers were significantly than in referents. The values of mean corpuscular volume (MCV), white blood cell counts (WBCs), neutrophils, lymphocytes, interferon-γ (IFN-γ), and IgG in the exposed workers were lower than in referents. Combined exposure to Ga, In, and As showed a significant change in trends of decreased MCV, WBCs, neutrophils, lymphocytes, IFN,γ, and IgG value, after adjusting for appropriate confounders.
Conclusion: These findings indicated that heavier exposed to Ga, In, and As may suppress hematological and immunological variables.

Keywords: *Light Emitting Diodes; Hematological; Immunological.*

1. Introduction

Light-emitting diodes (LEDs) manufacturing is a metallic-intensive optoelectronic industry. Toxic metals of group ⅢA and ⅤA of the periodic table are widely used in this industry, such as GaAs, InGaAs, and In GaAsP. These materials are used extensively in the operation of epitaxial growth, and dopant (Robinson 1983, Lay et al. 2002, Tanaka 2004).
In previous studies, occupational exposure to gallium (Ga), indium (In) , and arsenic (As) had been described significantly ($p < 0.05$) higher in exposed group than in referents (Liao et al, 2004. Chen, 2007). These metals or metalloids are known to alter heme synthesis (Woods et al. 1979, Aoki et al. 1990, Flora & Das Gupta 1992, Hatlelid et al. 1996), initiate apoptosis (Bustamante et al. 1997, Chang et al. 2003, Pyszel et al. 2005, Zhou et al. 2005). And cause carcinogenic effects (Fowler et al. 1993) in experimental studies. Overexposure to Ga and As have been shown to cause toxic effects of lipid peroxidation in optoelectronic workers (Liao et al. 2006). Exposure to In could cause interstitial lung damage in indium processing and indium compound production workers (Chonan et al. 2007, Hamaguchi et al. 2008). Luo et al suggested that leucopenia was a potential health effect in male fabrication workers in the semiconductor industry (Luo et al. 2002).
Taiwan is a preferred location for optoelectronic producing facilities; the optoelectronic production value has increased by 210 % from 2003 to 2007. Among the optoelectronic facilities, the production value of LED increased along with the expansion of the global photonics market, which has comprised the largest production (50%) of the global photonics market since 2005. For this reason, the number of LED companies and workers has been increasing rapidly. Hematopoietic synthesis and immunological response are general functions of humankind; detection of its abnormality in the blood cell reflects a widespread injury to other tissues (Piomelli 1981, Luster et al. 1993, Goering & Rehm 1990). Because workers in the optoelectronic factories are potential exposed to Ga, in, and As (Liao et al. 2004), their health may be at risk. The current study is undertaken to evaluate possible hematological and immunological effects of Ga, in, and as among the LED workers. To our knowledge, this is the first report to address the effects of optoelectronic metal in workers.

2. Methods

2.1. Subjects

We studied subjects from two LED factories in northern Taiwan. They were selected at the time of their annual medical examination between 2000 and 2003. Two workers were excluded because of past history of thyroid, and tumor diseases. Therefore, the final study population consisted of 91 LED workers who worked in the fabrication room (11 fabrication equipment preventative workers, 30 dopants and thin film workers, 25 fabrication supervisors and engineers, and 25 dice checking). And 24 referents (office workers) who worked in offices isolated from the fabrication rooms. Each worker included in the study population completed a questionnaire, including information about age, working duration (months), body mass index (BMI), sex, education level, cigarette smoking, alcohol consumption, regular intake of vitamins, and past history of disease. The study was approved by the Institutional Review Board in Kaohsiung Medical University. All participants were informed and signed consent forms.

2.2. Biological monitoring

Urine specimens and blood sample from the antecubital vein were obtained from the study subjects during their health check- ups in the morning. Subjects were instructed to eat nothing for at least 8 hours prior to blood and urine sampling. Blood and urine specimens were kept at -20oC prior to analysis (Pan 1993). Levels of heavy metals were determined by inductively coupled plasma-mass spectrometry after microwave dissolution, the detail procedures and quality assurance have been described elsewhere (Liao et al. 2004).

2.3. Determination of hematological and immunological variables

Venous blood was collected for blood routine and immunological tests. Blood (whole blood 3 ml in EDTA) was collected for measurinf hematological markers such as complete blood cell (CBC) tests, and WBC differential. Serum was taken from the supernatants of centrifuged blood. Serum tumor necrosis factor –α (TNF-α), interleukin - 1β (IL-1β), interferon-γ (IFN-γ), IgM, and IgG levels were determined by using commercial- ized ELISA kits (Antigenix America Inc, NY) according to manufacturer's instructions.

All blood samples were collected between 7:00 a.m. and 9:00 a.m. The collected samples were transported to a laboratory for analysis within six hours. The blood tests were analyzed in the laboratory of the Department of Clinical Pathology in the Jean Tide Medical Examination Center. Standard quality control was performed twice daily.

2.4. Statistics

All results were presented as the value of means, or median. When two groups were compared. The Student's test (normalized transformation) or Chi-square test were employed. The limit of significance was set at $p < 0.05$. The correlations in the various groups were studied by Pearson's regression after the data had been normalized. The data were also subjected to multiple linear regression models to examine the net effects and dose-response relation between exposure situation and hematological, and immunological outcomes. A multiple logistic regression model was used to determine the odd ratio (OR) of abnormal health effects on metals exposure after controlling for other independent variables. All analyses were performed with the SPSS program (SPSS Inc, Chicago, IL) for Windows.

3. Results

3.1. Distribution of demographic characteristic, and exposure biomarkers among study subjects

The demographic, and levels of metals in human's specimens are showed in table 1. The mean age (30.34 ± 18.20 years), levels of blood In (0.15 ± 0.09 ppb), urine Ga (0.34 ± 0.13 ppb), and urine As (25.94 ± 24.72 ppb) were significantly higher in the exposed workers than in the referents.

3.2. Hematological and immunological effects

Table 2 shows the mean of hematological and immunological parameters in the exposed group and referents. There were significantly lower value of MCV, WBC, neutrophils, lymphocytes, IFN-γ, and IgG in the exposed group than in referents. There were higher values of MCHC in the exposed group than in the referents. The frequency of abnormality with MCHC was significantly higher in exposed group than in referents (OR = 19.52, $P < 0.05$). There was not any difference between the exposed group and referents in the frequency of abnormality with MCV, WBCs, neutrophils, and lymphocytes.

Table 1: Subjects Characteristics, and Exposure Measures of Exposed Workers (N = 91) and Referents (N = 24).

Items	Exposure group	Referents
Age* (yrs)	30.34 ± 18.20	26.04 ± 5.98
Duration of employment (months)	30.16 ± 5.40	29.63 ± 15.15
Body mass index (kg/m2)	22.97 ± 4.32	23.90 ± 5.11
Sex		
male	52	14
female	39	10
Education level		
< college	68	20
≧college	23	4
Smoking status		
no	79	23
yes	12	1
Alcohol consumption		
no	77	23
yes	14	1
Vitamin complex intake*		
no	68	19
yes	23	5
Blood		
Ga	0.53 ± 0.35	0.43 ± 0.29
In*	0.15 ± 0.09	0.10 ± 0.08
As	6.85 ± 5.93	6.62 ± 6.63
Urine (ppb)		
Ga*	0.34 ± 0.13	0.17 ± 0.11
In	0.02 ± 0.03	0.01 ± 0.02
As*	29.94 ± 24.72	19.03 ± 18 .14

*X2 test: exposure group versus referents, df = 1, $p < 0.05$.

Table 2: Comparison of Hematological and Immunological Parameters between Exposed Group (N = 91) and Referents (N = 24).

Parameter	Exposure group	Referents
CBC differential		
RBC (m/μL) male	5.05 ± 0.53	5.02 ± 0.46
(low, normal, high)	(4, 37, 11)	(0, 5, 0)
female	4.35 ± 0.43	4.75 ± 0.55
(low, normal, high)	(9, 29, 1)	(3, 13, 3)
Hb (g/dL) male	14.99 ± 1.60	13.14 ± 1.98
(low, normal, high)	(9, 43, 0)	(3, 2, 0)
Female	13.15 ± 1.94	13.16 ± 1.48
(low, normal, high)	(8, 27, 4)	(4, 15, 0)
Hct (%) male	46.74 ± 3.06	45.50 ± 1.82
(low, normal, high)	(2, 50, 0)	(0, 5, 0)
female	39.21 ± 4.41	38.64 ± 3.56
(low, normal, high)	(9, 30, 0)	(8, 10, 1)
MCV* (%)	84.32 ± 7.68	89.77 ± 5.96
(low, normal, high)	(9, 79, 3)	(0, 23, 1)
MCH (pg)	29.75 ± 3.18	29.55 ± 2.74
(low, normal, high)	(8, 75, 8)	(3, 20, 1)
MCHC* (%)	35.49 ± 4.65	32.89 ± 1.67
(low, normal, high)	(8, 42, 41)	(3, 20, 1)
Platelet (k/μL)	245.34 ± 56.44	243.58 ± 40.0
(low, normal, high)	(3, 87, 1)	(0, 24, 0)
WBC* (k/μL)	5.82 ± 1.13	6.96 ± 1.54
(low, normal, high)	(20, 71, 0)	(1, 23, 0)
WBC differential (k/μL)		
Neutrophils*	3.02 ± 1.23	3.92 ± 1.34
(low, normal, high)	(15, 76, 0)	(1, 23, 0)
Lymphocytes*	2.12 ± 0.61	2.44 ± 0.77
(low, normal, high)	(1,90, 0)	(0, 24, 0)
Monocytes	0.32 ± 0.22	0.26 ± 0.19
(low, normal, high)	(0, 90, 1)	(0, 24, 0)
Eosinophils	0.30 ± 0.20	0.31 ± 0.19
(low, normal, high)	(0, 90, 1)	(0, 24, 0)
Basophils	0.06 ± 0.07	0.03 ± 0.05
(low, normal, high)	(0, 86, 5)	(0, 23, 1)
Immune markers		
TNF-α (pg/mL)	6.35 ± 0.79	6.43 ± 0.82
IL-1β (pg/mL)	3.18 ± 0.41	3.31 ± 0.37
IFN-γ* (pg/mL)	20.81 ± 3.83	25.49 ± 3.47
IgM (mg/dL)	87.92 ± 18.33	93.42 ± 21.94
IgG* (mg/dL)	1241.10 ± 199.59	1376.42 ± 206.23

*: Student's t test, significant difference, $p < 0.05$ (exposed workers vs. referents, df = 1, all data had been normalized).

3.4. Dose-response relation between biomarkers and hematological and immunological effects

Among the 91 LED workers, blood In, urine Ga and As levels were negatively and significantly correlated ($p < 0.05$) with MCV, WBCs, neutrophils, lymphocytes, IFN-γ, and IgG values respectively (table 3). Based on the analysis of hematological and immunological parameters among the 91 LED worker, Ga showed higher coefficients of determination for MCV (r 2= 0.49), WBC (r2 = 0.42), neutrophils (r2 = 0.26), lymphocytes (r2 = 0.44), IFN-γ (r2 = 0.26), and IgG (r2 = 0.69) values respectively than In, and As.

Among the 24 office workers, there was no significant correlation between any metal in blood or urine and hematological or immunological parameters in referents.

Table 3: The Pearson's Correlation Coefficient between Biomarkers and Hematological and Immunological Parameters.

	Exposure group	Referents	Total
Blood			
In vs MCV	-0.35*	0.03	-0.32*
In vs MCHC	0.02	-0.10	0.06
In vs WBCs	-0.39*	0.12	-0.31*
In vs neutrophils	-0.22*	0.32	-0.26
In vs lymphocytes	-0.23*	-0.19	-0.25*
In vs IFN-γ	-0.27*	0.13	-0.27*
In vs IgG	-0.51*	0.01	-0.43*
Urine			
Ga vs MCV	-0.70*	0.14	-0.63*
Ga vs MCHC	0.17	0.15	0.26*
Ga vs WBCs	-0.65*	0.07	-0.57*
Ga vs neutrophils	-0.51*	-0.08	-0.50*
Ga vs lymphocytes	-0.21*	0.26	-0.19*
Ga vs IFN-γ	-0.51*	0.29	-0.51*
Ga vs IgG	-0.83*	0.13	-0.68*
As vs MCV	-0.34*	0.09	-0.32*
As vs MCHC	-0.04	0.25	0.04
As vs WBCs	-0.25*	-0.23	-0.29*
As vs neutrophils	0.14	-0.38	-0.24
As vs lymphocytes	-0.26*	0.29	-0.17
As vs IFN-γ	-0.32*	0.15	-0.30*
As vs IgG	0.54*	0.14	-0.44*

*: significant difference, $p < 0.05$, (all data had been normalized).

3.5. Combined exposure to in, GA and as and hematological and immunological effects

In multiple regression analysis. Exposed workers affected negatively and significantly MCV ($\beta = -0.33$, $p < 0.05$), WBC ($\beta = -0.37$, $p < 0.05$), neutrophils ($\beta = -0.27$, $p < 0.05$), lymphocytes ($\beta = -0.25$, $p < 0.05$), IFN-γ($\beta = -0.52$, $p < 0.05$), and IgG ($\beta = -0.34$, $p < 0.05$) values than the office workers ($p < 0.05$) after adjusting for another factor, including duration of employment, BMI, sex, education level, cigarette smoking, alcohol consumption, regular intake of vitamins, and past history of disease (table4).

4. Discussion

This study showed an elevated blood. In, urine Ga, urine. As, blood MCHC, but decreased blood MCV, WBCs, neutrophils, lymphocytes, IFN-γ, and IgG values in workers exposed to Ga, In, and As when compared with the referents. Association analysis showed that blood. In, urine Ga, and urine. As levels had significant correlation with blood MCV, WBCs, neutrophils, lymphocytes, IFN-γ, and IgG in exposed workers respectively. Ga showed the highest coefficient of determination for the hematological and immunological parameters than In, and As. In multiple regression analysis, the dose-response relationships were found between the cumulative biological exposed dose and MCV, WBCs, neutrophils, lymphocytes, IFN-γ, and IgG. These results implied that heavier exposure to Ga, In , and As may suppress the hematological and immunological variables in the LED workers.

The finding of reduced MCV, WBCs neutrophils, and lymphocytes was consistent with other reports in the literatures (Luo et al. 2002, Heck et al. 2008). Several murine in vitro and in vivo studies have demonstrated the effects of Ga, In, and As on lymphocyte proliferation and on immunoglobulin production (Balaban et al. 1987, Gonsebatt et al. 1992, Huang et al. 1994, Drobyski et al. 1996, Gondre-Lewis et al. 2003). Chitambar et al suggested that transferring-gallium complex (Tf-Ga) capable of targeting Tf receptor-bearing T- and B- lymphocytes and interferes with their proliferation and function (Chitambar et al. 1989). The cytokines induced by helper T cells might not only opsonise the inflammatory systems but also affect the proliferative response of splenic B blast cells (Romaganani 1997, Chang et al. 2002). The results showed that at the counts (WBC, neutrophil, and lymphocyte) and levels (IFN-α, and IgG) tests, combined exposure to Ga, In, and As could impair the subtle lymphocyte stimulation and proliferation. We found exposed who were older workers could be protected from a decline of IFN-α, and IgG. The effects of age on IFN-α, and IgG were not known, and should be further investigated.

Table 4: Linear Regression Models of the Hematological and Immunological Parameters of Exposure Situation Adjusted for Potential Confounders Respectively (N = 115).

Variables	MCV	WBCs	Neutrophils	Lymphocytes	IFN-γ	IgG
Regression coefficient (β)						
Exposure (Referents = 0)	-0.33*	-0.37*	-0.27*	-0.35*	-0.52*	-0.34*
Duration of employment	0.11	-0.03	0.03	-0.05	0.08	0.09
Age (yrs)	0.08	0.10	0.05	0.11	0.23*	0.24*
BMI (kg/m2)	-0.10	-0.07	-0.08	0.00	-0.07	-0.07
Sex (male = 0)	-0.05	0.08	0.15	-0.17	-0.08	-0.10
Education level (< college = 0)	0.08	0.08	0.14	-0.09	0.02	-0.04
Smoking status (no = 0)	0.08	0.15	0.08	0.10	0.02	-0.00
Alcohol consumption (n = 0)	-0.13	-0.13	-0.04	-0.15	-0.10	-0.11
Vitamin complex intakes (no = 0)	0.16	0.03	0.09	-0.21	-0.08	0.02

P value < 0.05, significant difference. All data had been normalized.

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