Elevated red blood cell distribution width and inflammation in printing workers

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Background: The aim of this study was to estimate the effects of exposure to chemical compounds on systemic biochemical inflammatory markers in printing industry workers.

Material/Methods: Fifty-eight printing workers from 19 different small- and medium-sized enterprises in the printing sector were investigated. For comparison, 80 healthy workers not subjected to workplace chemicals served as control subjects.

Results: No significant differences were observed between the printing workers and control subjects with respect to age, BMI, waist circumference/hip circumference ratio, smoking, and alcohol consumption. Printing workers had significantly higher serum TNF-alpha levels (11.02±5.34 vs. 9.26±3.87 pg/ml, p=0.039), plasma fibrinogen levels (1.74±0.49 vs. 1.38±0.5 mg/dl, p=0.012), and red blood cell distribution width (RDW-SD) (49.77±3.09 vs. 47.3±2.88 p<0.01) compared to control subjects.

Conclusions: Elevation of RDW, serum TNF-alpha, and plasma fibrinogen levels in printing workers may be due to systemic toxic effects of chemical compounds used in this sector. TNF-alpha is an inflammatory cytokine that has a wide spectrum of biological activities, and fibrinogen plays an important role in pathological processes. Some compounds may be carcinogenic or mutagenic. Better designed workplaces and working conditions will help to reduce the hazardous effects of chemical compounds.

Key words: red blood cell distribution width • tumor necrosis factor-alpha • fibrinogen • printing workers

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**Background**

Printing workers have traditionally high occupational exposure to health hazards [1–4]. There are significant health risks, such as dermatitis, musculoskeletal disorders, occupational asthma, deafness, eye damage, and other problems associated with the use of solvents and other chemical compounds that need to be considered in the printing sector [1].

Cleaning solvents, fixer solutions, deletion fluids, and various inks are used extensively in this sector. They contain corrosive acids (e.g., concentrated nitric and sulfuric acids, hydrofluoric acid), strong alkalis (e.g., concentrated sodium or potassium hydroxide), hydroquinone, reactive acrylates, sodium thiosulphate, dilute formaldehyde solution, dichromates (e.g., ammonium, potassium and sodium dichromates), isopropyl alcohol, methyl ethyl ketone, white spirit, alcohols (e.g., industrial methylated spirits), esters (e.g., ethyl acetate), aromatic hydrocarbons (e.g., toluene, xylene), propanol, isocyanate pre-polymers, perchloroethylene, N-vinyl pyrrolidone, chlorinated hydrocarbons (e.g., dichloromethane), and ketones (e.g., cyclohexanone). Exposure to such chemicals can have acute or delayed effects. Solvents and other chemical compounds can be taken into the body by breathing them in, passing through the skin, or by eating food touched by contaminated hands. Some compounds may be carcinogenic or mutagenic [1].

Research continues to reveal systemic and local inflammatory effects of these compounds [5–11]. There is no data about the effects of these agents on systemic inflammatory markers in printing workers. This study was designed to determine the systemic effects of exposure to chemicals in printing industry workers.

**Material and Methods**

Fifty-eight printing workers from 19 small- and medium-sized printing enterprises were investigated (Group I). For comparison, 80 healthy age-matched workers in fields other than printing, not subjected to workplace chemicals, were chosen and served as control subjects (Group II). All of the printing workers and controls are given in Table 1 and 2. Mean ages of Group I and Group II were 26.0±8.9 years (15–49) and 27.01±6.26 years (18–50), respectively. All workers gave informed consent and the study protocol was approved by the local ethics committee. All subjects were examined physically. Age, height, weight, hip and waist circumferences, alcohol consumption, and smoking status were recorded. Blood samples were obtained from the ante-cubital veins at the end of the work shift. Samples were promptly centrifuged at 2500 g, at 4°C, for 10 minutes. Serum and plasma samples were aliquoted and saved at −80°C until measurement of TNF-alpha, plasma fibrinogen levels, and other biochemical parameters.

**Biochemical analysis**

Routine CBC (complete blood count) tests were promptly conducted with Sysmex XT-2000i automated hematology analyser (Sysmex Corporation of America). Plasma fibrinogen levels were measured using the Clauss clotting method (STA-Fibrinogen Diagnostica Stago) with the STA Compact automated coagulation analyzer (Diagnostica Stago, Albio, France). Serum levels of TNF-alpha were determined using a chemiluminescence enzyme immunometric assay (Immulite-One, Immunoassay Analyzer; Immulite DPC, Los Angeles CA, USA). Serum glucose, urea, creatinine, total, LDL and HDL cholesterol, triglyceride, ALT, AST, and total and direct bilirubin levels were measured on a Roche Hitachi Modular System (Mannheim, Germany) autoanalyzer with spectrophotometer.

**Statistical analysis**

Data were analyzed in SPSS Program 15.0 (SPSS Inc, Chicago, IL, USA). For comparison, Student t test, chi-square test, and Mann-Whitney U test were used as appropriate. For correlations, Pearson’s and Spearman’s correlation tests were used. Statistical significance was assumed when the p-value was less than 0.05. Results are expressed as the mean ±SD and percent.

**Results**

Demographic, hematological and biochemical data of workers and controls are given in Table 1 and 2. Mean ages of Group I and Group II were 26.0±8.9 years (15–49) and 27.01±6.26 years (18–50), respectively. All workers had been working at least for 1 year in this occupation (mean=11.2±9.05 years).
### Table 1. Demographic data of printing workers and control subjects

| Parameters          | Printing workers (n=58) | Control subjects (n=80) | P value |
|---------------------|-------------------------|-------------------------|---------|
| Age (year)          | 25.72±8.43              | 27.33±6.60              | 0.209   |
| BMI (kg/cm²)        | 23.89±3.99              | 25.17±3.93              | 0.066   |
| Waist circ/hip circ (cm/cm)| 0.89±0.08 | 0.89±0.06              | 0.998   |
| Alcohol consumption (%) | 22.7                  | 16.7                    | 0.652   |
| Smoking (pockets/year) | 157.15±176.70         | 124.32±199.81           | 0.331   |
| Working duration (year) | 11.23±9.05               | –                       |         |

Table 1 was obtained from independent t test and chi-square test, the difference is significant if p value <0.05. BMI – Body-mass index.

### Table 2. Hematological and biochemical data of printing workers and control subjects

| Parameters          | Printing workers (n=58) | Control subjects (n=80) | P value |
|---------------------|-------------------------|-------------------------|---------|
| WBC (10³/µL)        | 6.60±1.27               | 6.87±1.41               | 0.298   |
| RBC (10⁶/µL)        | 5.07±0.52               | 5.22±1.28               | 0.458   |
| Hemoglobin (g/dl)   | 14.93±1.07              | 15.12±1.20              | 0.377   |
| Hematocrit (%)      | 44.64±3.72              | 43.99±3.75              | 0.354   |
| MCV (fl/cell)       | 88.73±4.41              | 85.76±8.43              | 0.076   |
| RDW-SD              | 49.77±3.09              | 47.3±2.88               | <0.001  |
| PLT (10³/µL)        | 240.08±45.71            | 224.06±47.65            | 0.068   |
| MPV (fl)            | 9.77±1.05               | 9.46±0.99               | 0.099   |
| Glucose (mg/dL)     | 86.59±11.06             | 88.45±8.69              | 0.292   |
| Urea (mg/dL)        | 15.22±7.43              | 17.94±7.20              | 0.060   |
| Creatinine (mg/dL)  | 0.81±0.12               | 0.85±0.11               | 0.053   |
| ALT (U/L)           | 21.69±13.74             | 25±14.14                | 0.186   |
| AST (U/L)           | 20.52±5.78              | 21.18±5.75              | 0.508   |
| ALP (U/L)           | 83.48±31.82             | 73.07±28.90             | 0.083   |
| Total Cholesterol (mg/dL) | 163.90±29.34        | 170.88±33.03            | 0.220   |
| Triglyceride (mg/dL)| 137.45±83.67            | 119.14±64.63            | 0.169   |
| HDL-C (mg/dL)       | 37.54±7.80              | 39.16±7.75              | 0.242   |
| LDL-C (mg/dL)       | 99.12±23.05             | 107±27.51               | 0.089   |
| Total Bilirubin (mg/dL) | 0.80±0.44           | 0.81±0.33               | 0.968   |
| Direct Bilirubin (mg/dL) | 0.26±0.13            | 0.27±0.12               | 0.762   |
| Uric Acid (mg/dL)   | 5.77±0.94               | 5.80±1.15               | 0.851   |
| TNF-alpha (pg/mL)   | 1.02±3.54               | 9.26±3.87               | 0.039   |
| Fibrinogen (g/L)    | 1.74±0.49               | 1.38±0.5                | 0.012   |

Table 2 was obtained from independent t test, the difference is significant if p value <0.05. ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate amino transferase; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; MCV – mean corpuscular volume; MPV – mean platelet volume; PLT – platelet; RBC – red blood cell; RDW-SD – red cell distribution width-standard deviation; WBC – white blood cell; TNF – tumor necrosis factor.
No difference was detected with respect to age, BMI, waist circumference/hip circumference ratios, smoking, and alcohol usage between Group I and Group II (p>0.05) (Table 1). For hematological and biochemical analyses, no difference was determined between groups with respect to WBC and RBC MCV, MPV, PLT, and routine biochemical tests. However, Group I had significantly higher red blood cell distribution width (RDW-SD) values (49.77±3.09 vs. 47.3±2.88, p<0.05) (Table 2).

Serum TNF-alpha levels (11.02±5.34 vs. 9.26±3.87 pg/ml, p=0.039), and plasma fibrinogen levels (1.74±0.49 vs. 1.38±0.5 mg/dl, p=0.012) were compared to Group II.

**Discussion**

We found that RDW-SD was statistically higher in printing workers than control subjects (p<0.01). This finding may be evaluated as anisocytosis. RDW is a measurement of the size variation, as well as an index of the heterogeneity of the erythrocytes (i.e., anisocytosis). Higher RDW values reflect greater variation in RBC volumes and were found to be related to many diseases in previous studies [12–14].

We found that RBC and WBC were lower and Hb and MCV were higher in Group I, even if differences were not significant. We hypothesize that DNA and cell division might have been affected by exposure to chemicals via cytotoxicity. Studies should be designed to verify this hypothesis. In addition, in vitro inhibition of human erythroid colony formation by TNF-alpha was reported [15]. It has been demonstrated that RDW values are associated with inflammatory markers [16]. In our study, printing workers had higher TNF-alpha and RDW-SD. However, there was no correlation between TNF-alpha and RDW in each of groups and in all subjects.

Peng et al. had found that RDW was higher in the workers occupationally exposed to lead than in an unexposed group, and blood lead was weakly positively correlated with RDW [17]. It has been reported that RDW is higher in prehypertensive and hypertensive patients compared with healthy controls [18]. It has been shown that RDW are elevated in cardiovascular disease pulmonary disease, liver disease, stroke, peripheral artery disease, inflammatory bowel disease, colon cancer, and neoplastic metastases to the bone marrow [19–27]. Many studies have identified RDW as a predictor of all-cause and cardiac mortality [28–30].

Kurtoglu et al. have reported that mean RDW values are higher in smokers than in nonsmokers. They identified significant positive correlations between RDW and number of cigarettes smoked per day and between RDW and duration of smoking [31]. When we excluded smokers, RDW was higher in workers than in controls.

In our study, we found higher TNF-alpha levels in printing workers, possibly due to chronic stress arising from toxic exposure to chemicals. TNF-alpha is synthesized in many tissues with proinflammatory properties and it regulates synthesis of acute-phase reactants such as fibrinogen and factor VII [32]. It inhibits anticoagulatory mechanisms and promotes thrombotic processes and therefore plays an important role in pathological processes such as venous thromboses, arteriosclerosis, vasculitis, and heart failure [33, 34]. It has a direct cytotoxic effect, modulates cell growth and differentiation, and plays a role in chronic inflammatory conditions [35].

It has been found that the concentration levels of inflammatory biomarkers and eosinophilic cationic protein level in the lavage of painters were higher than in the control group. It has been reported that inhalation of VOCs (volatile organic compounds) could be responsible for the occurrence of respiratory inflammatory and allergic diseases [36].

In our study, we found fibrinogen was elevated. It is a key dimeric glycoprotein, taking part in the production of acute-phase reactants by the liver. Fibrinogen levels become elevated with tissue inflammation or tissue destruction. High plasma fibrinogen level may underlie many disorders [37–40].

In this study, printing workers had been working for 11 years on average, ranging from 1 to 30 years. When we classified the workers according to their years worked (less than 5 years, 5–10 years, 10–15 years, 15–20 years, and more than 20 years), the non-significant parameters did not gain statistical significance (p>0.05). No statistical correlation between years worked and TNF-alpha, fibrinogen, and RDW-SD was found in our study.

**Conclusions**

We found systemic effects of chemicals used in the printing sector and an association between TNF-alpha, fibrinogen, and RDW in printing workers. Working in this profession may contribute to the burden of inflammation and many diseases in printing workers. Improved use of personal protective equipment to reduce occupational exposure to toxic chemical may be indicated by this study.

Future studies in large-scale printing workplaces with larger numbers of workers are required to elucidate this issue.
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