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USING CD24/CD11B FOR BIOMONITORING OF WORKERS EXPOSED TO CARCINOGENIC CHEMICALS IN METAL COATING PROCESSES

BY

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Abstract

Introduction: Surface coating processes have been developed to provide corrosion-resistance, increased durability for metallic products such as pipelines. Bisphenol A, bisphenol F, and phenolic novolac are the most widely used epoxy resins. Previous studies found that bisphenol A increased proliferation of acute myeloid leukaemia (AML) cells and led to the development and progression of lymphoma due to DNA damage. Blood levels of CD24 together with CD11b (Cluster of Differentiation 24 and 11b) have been introduced as promising screening tests for early diagnosis of hematologic malignancies. **Aim of Work:** To evaluate the use of blood CD24/CD11b for early detection of hematological malignancies among workers exposed to epoxy resins containing Bisphenol A in metal coating processes. **Materials and Methods:** This cross-sectional study included 36 exposed workers, 36 administrative employees, and 36 patients with haematological malignancies. All groups underwent history, clinical examination, complete blood picture (CBC), CD24/CD11b blood level and urinary bisphenol A (BPA). **Results:** There was a high level of urinary BPA, CD24, CD11b, and CD24/Cd11b among the exposed and the haematological malignancies groups. The group of haematological malignancies had lower haematological parameters. The exposed group had significantly lower haemoglobin, MCV, MCH and MCHC. The urinary BPA showed positive correlation with the exposure duration, CD24 and CD11b. The exposure duration showed positive correlation with CD24. **Conclusion:** Workers exposed to epoxy resins used for metal coating had a significantly higher urinary level of BPA , higher blood levels of CD24, CD11b and the CD24/Cd11b ratio, which indicated increased risk for hematological malignancies. **Recommendations:** The blood levels of CD24 and CD11b are recommended as biological markers for early detection of haematological malignancies among workers exposed to BPA in epoxy resins.

Keywords: Epoxy resins; Bisphenol A; CD24 (Cluster of Differentiation 24); CD11b(Cluster of Differentiation 11b) and Haematological malignancies.

Introduction

Surface coating processes have been developed to provide corrosion-resistance, increased durability for metallic products exposed to stressful environmental conditions (Huang et al., 2023, Nazarzade et al., 2023).

Epoxy-based coating is commonly used in pipelines for protection against corrosion. Examples include oil-gas pipelines, water and/or wastewater pipelines, and concrete pipelines. Bisphenol A, bisphenol F, and phenolic novolac are the most used epoxy resins (Ramakrishnan et al., 2022).

A previous study found that bisphenol A increased the cells proliferation in acute myeloid leukemia (AML) and decreased their sensitivity to chemotherapy (Zhang et al., 2020). Evidence from multiple studies on solid malignancies and acute myeloid leukaemia emphasizes the effect of bisphenol A exposure on malignancy progression through particular genes which are implicated in malignant cell persistence (Nomiri, et al., 2019). Exposure to bisphenol A damages the DNA and affects the immune system. The lymphoid cells with damaged DNA result in the initiation and progression of lymphoma, especially in immuno-compromised patients (Chen et al., 2021).

In hematologic malignancies, early detection may significantly increase the response to treatment and improve the prognosis. Early detection of leukaemia may give a cure rate up to 80% (Llano, 2016). Similarly, the early detection of lymphoma is an important determinant of better prognosis (ACS, 2019). Unfortunately, there is no reliable single screening test to detect the haematological malignancies at early stages before the appearance of clinical manifestations. Moreover, there are no gold standard methods for screening the high-risk groups regularly (Hussaini, 2015).

CD24 is a glycosylated mucin-like, phosphoinositol-attached membrane protein. Increased levels of CD24 were detected in several malignancies, especially hematological malignancies, and have been related to poor prognosis (Fischer et al., 1990). Blood levels of CD24 together with CD11b have been introduced as promising screening tests for early diagnosis of hematologic malignancies (Shapira et al., 2021[b]). CD11b is the α-subunit of the β2 (CD18) integrin adhesion molecule, expressed on the myeloid cells' surface

(Ahn et al., 2010). CD11b is involved in cell adhesion and migration through the endothelium or epithelium, and recruitment to inflamed cells and tumours (Schmid and Varner, 2012).

There is currently a research gap in studying the application of CD24 and CD11b as a screening tool in healthy population at-risk to develop haematological malignancies. That is why this research was done.

Aim of Work

To evaluate the use of blood CD24/CD11b for early detection of haematological malignancies among workers exposed to epoxy resins containing Bisphenol A in metal coating processes.

Materials and Methods

Study design: This is a cross-sectional comparative analytical study.

Place and duration of the study: The study took place in a steel pipeline factory in Helwan, Cairo, from June to August 2024.

Study Sample: The study included an exposed group of 36 workers (exposed to epoxy resins used in the metal coating process) in a steel pipelines factory in Helwan, Cairo. The control group included 36 non-exposed administrative employees in the student and post-graduate affairs as well as the human resources departments, in the Faculty of Medicine, Cairo University. They all denied exposure to any chemicals, fumes, or vapors. A third comparison group included 36 patients currently diagnosed as having haematological malignancies and admitted to the internal medicine department of haematology at Cairo University Hospitals.

Sample size:

A sample size of 108 participants (36 per group) was calculated by G power program using one way analysis of variance based on the following statistical assumption: effect size f is 0.3 at 80% power and 0.05 significant levels. The study groups included: exposed group (workers exposed to epoxy resins for metal coating), a group of patients with haematological malignancies, and a non-exposed (control) group.

Inclusion criteria:

- Exposed group: All exposed workers were males, who have been working in the metal coating department of the pipeline manufacturing facility for more than one year.

- Control group: Administrative male employees not previously exposed to any chemicals, fumes of vapours.

- Malignancy group: Male patients, aged 20 or more years old, diagnosed with any type of haematological malignancy, admitted to the department of Internal Medicine and Clinical Haematology.

Exclusion criteria:

-Exposed group: Workers with other exposures from other jobs, and workers with known autoimmune diseases, or any type of malignancy.

-Non-exposed group: Female employees, and employees with known autoimmune diseases, or any type of malignancy.

-Malignancy group: Female patients, and patients less than 20 years old.

Study methods:

All participating individuals were subjected to:

- A questionnaire including personal, occupational, medical and family histories.
- Clinical examination.
- Laboratory investigations included:

1. Complete blood count (CBC).

2. CD24/CD11b level in the peripheral blood: using human CD24 and human Integrin alpha M (CD11b) ELISA kit purchased from ELK Biotechnology.

3. Bisphenol A in urine: using Bisphenol-A ELISA kit purchased from ELK Biotechnology.

A blood sample of 8 ml was obtained from each subject, privately inside the facility clinic, through venipuncture from the arm using a disposable syringe, using aseptic techniques. Each sample was divided into two tubes: one tube contained dipotassium-EDTA for complete blood picture (CBC), and the other tube was left for 2 hours at room temperature to clot and then centrifuged for 20 minutes for serum separation to measure CD24/CD11b. The separated serum was stored at -20^{o}C until analysed later.

A midstream urine sample was collected from each participant privately using sterile containers for measuring urinary Bisphenol A. The urine samples were centrifuged at the laboratory to remove particulates and were stored at -20^{o}C till the time of analysis.

All the laboratory tests were done at the Clinical Pathology laboratories of Cairo University Hospitals.

Consent

A written informed consent was voluntarily obtained from each participant, after a proper clarification of the objectives of the study. Participants' privacy and confidentiality were a priority during collection of samples, coding, and laboratory work. Participants were provided with copies of the results of their tests for further investigations and management if needed.

Ethical Approval

The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Cairo University, Egypt (Approval No.: N-91-2024). Approval of the factory administration was also obtained.

Data Management

Pre-coded data statistically analysed using the statistical package of social science software program version 21 (SPSS 21). Quantitative variables were expressed using mean and standard deviation (SD). One way ANOVA test was used to compare quantitative variables between more than two groups. Pearson Correlation coefficient (Pearson's r) was done to detect the linear relations between quantitative variables. P-value less than 0.05 was considered statistically significant and p-value less than 0.001 was considered highly statistically significant.

Results

The mean age of the exposed group, control group, and hematological malignancy group was 35.5±14 years, 31.4±6 years, and 34.6±13 years, respectively, with no statistically significant difference between the studied groups (p-value was >0.05 using ANOVA test), implying acceptable matching between the studied groups (not tabulated).

Table (1): Comparison of urinary level of bisphenol A among the studied groups.

Variables	Studied Groups	Mean ± SD	f	p-value	Post Hoc test p-value
Bisphenol A (ng/ml)	**a. Control group**	1.06 ±0.34	80.34	**< 0.001****	**a,b groups <0.001**** a,c groups >0.05 **b,c groups <0.001****
	b. Exposed group	2.14 ± 0.56			
	c. Haematological malignancy group	1.11 ± 0.28			

**: Highly statistically significant

Table (1) showed that the urinary level of Bisphenol A was significantly higher among the exposed group compared to both the control group and the group with haematological malignancies. No statistically significant difference was observed between the control group and the group with haematological malignancies.

Table (2): Comparison of blood level of CD24, CD11b and CD24/CD11b ratio among the studied groups.

Variables	Studied groups	Mean ± SD	f	p-value	Post Hoc test p-value
CD24 (ng/ml)	**a. Control group**	0.15 ± 0.52	26.23	**<0.001****	**a,b groups <0.05* a,c groups <0.001** b,c groups <0.001****
	b. Exposed group	0.38 ± 0.12			
	c. Haematological malignancy group	0.92 ± 0.80			
CD11b (pg/ml)	**a. Control group**	1001.17± 242.16	62.73	**<0.001****	**a,b groups <0.05* a,c groups <0.001** b,c groups <0.001****
	b. Exposed group	1248..83 ± 266.84			
	c. Haematological malignancy group	2115.55 ± 657.82			
CD24/ CD11b	**a. Control group**	0.16 ± 0.12	15.80	**<0.001****	**a,b groups <0.001** a,c groups <0.001**** b,c groups >0.05
	b. Exposed group	0.31 ± 0.20			
	c. Haematological malignancy group	0.34 ± 0.13			

*: Statistically significant **: Highly statistically significant

Table (2) showed that the blood levels of CD24 and CD11b were significantly higher among the exposed group and the haematological malignancy group compared to the control group. Also, the blood levels of CD24 and CD11b were significantly higher among the haematological malignancy group compared to both the exposed and control group. The CD24/CD11b ratio was significantly higher among the exposed and the haematological malignancy group compared to the control group. However, no statistically significant difference was found in the CD24/CD11b ratio among the haematological malignancy group compared to the exposed group.

Table (3): Comparison of blood indices values among the studied groups.

Blood Indices	Studied Groups	Mean ± SD	f	p-value	Post Hoc test p-value
RBC Count (million/mm^3)	**a. Control group**	4.84 ± 0.45	36.32	**<0.001****	a,b groups >0.05 **a,c groups <0.001**** **b,c groups <0.001****
	b. Exposed group	5.09 ± 0.53			
	c. Haematological malignancy group	3.76± 0.99			
Haemoglobin (g/dL)	**a. Control group**	1.63 14.49	45.14	**<0.001****	**a,b groups <0.05* a,c groups <0.001**** **b,c groups <0.001****
	b. Exposed group	1.39 13.43			
	c. Haematological malignancy group	2.24 10.26			
Haematocrit (%)	**a. Control group**	4.96 42.80	45.19	**<0.001****	a,b groups >0.05 **a,c groups <0.001**** **b,c groups <0.001****
	b. Exposed group	2.65 40.15			
	c. Haematological malignancy group	7.35 30.23			
Mean Cell Volume (MCV) (fL)	**a. Control group**	5.92 86.30	10.46	**<0.001****	**a,b groups <0.05* a,c groups <0.001**** **b,c groups <0.001****
	b. Exposed group	6.75 82.76			
	c. Haematological malignancy group	4.77 79.93			
Mean Cell Haemoglobin (MCH) (pg)	**a. Control group**	3.90 30.56	8.33	**<0.001****	**a,b groups <0.05* a,c groups <0.001**** **b,c groups <0.001****
	b. Exposed group	2.7 28.52			
	c. Haematological malignancy group	2.1 27.71			

Mean Cell Haemoglobin Concentration (MCHC) (g/dL)	**a. Control group**	2.06 32.50	32.55	**<0.001****	**a,b groups <0.001** a,c groups <0.001** b,c groups <0.05***
	b. Exposed group	1.56 34.48			
	c. Haematological malignancy group	2.34 30.64			
Platelet Count (Thousands/ mm^3)	**a. Control group**	246 16.46	5.15	**<0.05***	a,b groups >0.05 **a,c groups <0.05* b,c groups <0.05***
	b. Exposed group	16.85 234			
	c. Haematological malignancy group	17.30 169			
Total Leukocytic Count (TLC) (Thousands/ mm^3)	**a. Control group**	2.84 6.41	15.45	**<0.001****	a,b groups >0.05 **a,c groups <0.001** b,c groups <0.001****
	b. Exposed group	1.46 6.29			
	c. Haematological malignancy group	2.26 3.78			

*: Statistically significant **: Highly statistically significant

Table (3) showed that all the blood indices were significantly lower among the haematological malignancy group compared to both the exposed group and the control group. Also, significantly lower haemoglobin, MCV, MCH and MCHC were found among the exposed group compared to the control group. No statistically significant difference was found between the exposed and control group as regards the RBC count, haematocrit, platelet count or TLC.

Table (4): Correlation between the urinary level of Bisphenol A, and each of the duration of exposure, the blood level of CD24, the blood level of CD11b and the CD24/CD11b ratio among the studied group.

Variables	**Urinary level of Bisphenol-A**	
	r	**p-value**
Duration of exposure (Years)	0.32	**<0.05***
CD24 (ng/ml)	0.03	**<0.05***
CD11b (pg/ml)	0.33	**<0.05***
CD24/CD11b	0.20	>0.05

*: Statistically significant

Table (4) showed that a statistically significant positive correlation was found between the urinary level of Bisphenol-A and each of the duration of exposure, the blood level of CD24, and the blood level of CD11b. Nevertheless, no statistically significant correlation was found between the urinary level of Bisphenol-A and the CD24/CD11b ratio among the exposed group.

Table (5): Correlation between the duration of exposure to Bisphenol A and each of the blood level of CD24, the blood Level of CD11b and the CD24/CD11b ratio among the studied group.

Variables	Duration of Exposure to Bisphenol-A	
	r	p-value
CD24 (ng/ml)	0.69	**<0.001****
CD11b (pg/ml)	0.27	>0.05
CD24/CD11b	0.11	>0.05

**: Highly statistically significant

Table (5) showed that a highly statistically significant positive correlation was found between the duration of exposure to Bisphenol-A and the blood level of CD24 among the exposed group. However, no statistically significant correlation was observed between the duration of exposure to Bisphenol-A and either of the level of CD11b or the CD24/CD11b ratio among the exposed group.

Discussion

Epoxy resin is one of the commonly used industrial finishing materials, with multiple applications like pipeline coating and floor sealing (Vertuccio et al., 2018). Bisphenol A (BPA) is one of the most widely used epoxy resins (Ramakrishnan et al., 2022). Previous studies reported that CD24/CD11b marker in the blood might act as a screening tool for the early diagnosis of malignancy among the healthy individuals ([b]Shapira et al., 2021).

The present study aimed at evaluating the use of CD24/CD11b testing in peripheral blood as a screening tool for early diagnosis of haematological malignancies among workers exposed to epoxy resins used for metal coating.

The study included 108 participants, who were divided in 3 groups: the exposed group, the control group and the group with haematological malignancies. Each group consisted of 36 participants. The three groups were all non- smoker males and were matched as regards their age.

The current study showed that the urinary level of Bisphenol-A (BPA) was significantly higher among the exposed group compared to both the control group and the group with haematological malignancies (Table 1). This was consistent with a previous study where serum BPA concentrations were higher among the exposed male workers compared to the non-exposed ones (Zhuang et al., 2015).

The blood levels of CD24 were significantly higher among the exposed group compared to the control (Table 2). Exposure to BPA has been associated with increased risk of malignancy (Gao et al., 2015). Also, BPA can trigger the proliferation of cancer cells and increase their migration and invasion capabilities (Zhang et al., 2020). Several studies showed that the increased level of CD24 is associated with the early stages of tumour formation (Kraus et al., 2015, Altevogt et al., 2020). CD24 level has been related to the tumorigenicity of cancer stem cells (CSCs) in solid tumours as well as haematological malignancies. CSCs are the initiating precursor cells in the tumour development, and the relationship between CD24 and CSCs has been reported in multiple types of malignancy (Wang et al., 2024). Several studies found that CD24 positive cells have a high potential for tumour

formation (Lee et al., 2011 and Rostoker et al., 2015).

The blood level of CD24 among the studied group was significantly higher among the haematological malignancy group compared to both the exposed and control group (Table 2). This was in agreement with the study done by [b]Shapira et al., 2021; in which high levels of CD24 were found in patients with multiple myeloma, chronic lymphoid leukaemia (CLL) and non-Hodgkin lymphoma compared to healthy individuals. Previous studies showed that CD24 has been associated with tumour growth, invasion, metastasis, and has been proposed as a promising biomarker for prognosis of malignancy and response to chemotherapy (Altevogt et al., 2020). Also, high CD24 was considered as a marker of a poor prognosis in haematological malignancies (King et al., 2012).

The blood level of CD11b in the studied group was significantly higher among the exposed and the haematological malignancy group compared to the control group. Also, the blood level of CD11b was significantly higher among the haematological malignancy group compared to both the exposed and control group (Table 2). These findings were in harmony with the results of the study done by Zhang and his colleagues in 2015, which revealed that CD11b encourages myeloid cell movement to the blood, spleen and tumour microenvironment. CD11b can bind to various ligands, affecting different functions of myeloid cells, e.g. migration, adhesion, proliferation and phagocytosis. CD11b can therefore be used to modify the myeloid cells and produce T-cell tumour-reactive responses to defeat the difficulties in immunotherapy (Roche et al, 2023).

CD24/CD11b ratio was significantly higher among the exposed and the haematological malignancy group compared to the control group (Table 2). These findings agreed with the study done by [b]Shapira et al., 2021, in which the authors reported that a significantly higher CD24/CD11b ratio was detected in patients diagnosed with haematological malignancies compared to healthy individuals .

No statistically significant difference was found in the CD24/CD11b ratio among the haematological malignancy group compared to the studied exposed group (Table 2). This finding raises the possibility that a considerable number of exposed

workers might be in a very early asymptomatic phase of malignancy, or at high risk to develop malignancy.

[a]Shapira et al., 2021 reported that CD24/CD11b levels among healthy individuals may be used as a screening tool for the early diagnosis of malignancy among healthy individuals .

The blood indices among the studied group were significantly lower among the haematological malignancy group compared to both the exposed and the control groups (Table 3). This was partly in accordance with the results of Ebrahim and his colleagues in 2022, who reported that leucocytosis, anaemia, and thrombocytopenia were common in haematological malignancy patients. However, they also reported that low WBC count was found among 33% and 25% of patients with acute myeloid leukaemia and multiple myeloma respectively. The low WBC count in the current study can be caused by chemotherapy administered to the patients during the study, as chemotherapy can negatively affect bone marrow haematopoiesis leading to decreased WBCs production (Adeel et al., 2024).

Significantly lower haemoglobin, MCV, MCH and MCHC were found among the studied exposed group compared to the control one (Table 3). This mostly agreed with the results obtained by a previous Egyptian study were the authors found that there was a statistically significant lower haemoglobin level, RBCs count, haematocrit value and MCHC, and a non-significant lower platelet count among workers exposed to painting chemicals, compared to the control subjects (Abdel Maksoud et al., 2018).

There was a statistically significant positive correlation between the urinary level of Bisphenol-A and each of the duration of exposure, the blood level of CD24, and the blood level of CD11b among the exposed group. Nevertheless, no statistically significant correlation was found between the urinary level of Bisphenol-A and the CD24/CD11b ratio among the exposed group (Table 4). Also, a highly statistically significant positive correlation was found between the duration of exposure to Bisphenol-A and the blood level of CD24, but neither CD11b nor the CD24/CD11b ratio among the exposed group (Table 5).

There were no previous studies with similar correlations. However, those results agreed with the results of Guo

and co-workers in 2020; who reported that exposure to organic pollutants, such as BPA can increase the CD24 stem-like cells with the increased expression of markers related to the tumour initiation.

These results were also in concurrence with a previous study on immune cell profiles associated with measured exposure to phthalates (which is an endocrine disruptor similar to BPA). The authors found that the group of participants with phthalate exposure, CD11b expression in natural killer (NK) cell subsets was higher among the exposed group evidenced by high urinary phthalates level (Nygaard et al., 2021).

Conclusion and Recommendations

Workers exposed to epoxy resins used for metal coating had a significantly higher urinary level of BPA , higher blood levels of CD24, CD11b and the CD24/Cd11b ratio, which indicated increased risk for hematological malignancies, as these biomarkers were significantly increased among the hematological malignancies group compared to the control group. Also, significantly lower hemoglobin, MCV, MCH and MCHC were found among the exposed group compared to the control group. The urinary level of Bisphenol A had a positive correlation with each of the duration of exposure and the blood levels of CD24 and CD11b among the exposed group. Also, the duration of exposure to Bisphenol A had a positive correlation with the blood level of CD24 among the exposed group. The blood levels of CD24 and CD11b are recommended as biological markers for early detection of hematological malignancies among workers exposed to BPA in epoxy resins.

Conflict of Interest

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