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Evaluation of prevalence of eyes, skin symptoms and sCD8, sCD86 markers in wheat flour mill workers

Zaid Makki Mohammed AL-Hakkak Dr.
Ecology department, Faculty of science , University of Kufa, Iraq

Abstract
The aim of the study was to determine prevalence of eyes , skin symptoms and sCD8,sCD86 markers in wheat flour mill workers. The study included determine of eyes and skin symptoms using questionnaire in 80 male non-smoking wheat flour mill workers aged between 18-48 years (mean age 34.01±8.17) years with a mean duration of exposure (8.7±4.23) years and 70 non-smoking unexposed healthy subjects of the same age, and body mass index group served as control. The present work also examined serum levels of sCD8,sCD86 markers in wheat flour mill workers for 48 non-smoker male flour mill workers and 37 unexposed non-smoker individuals subjects as control group.
The results showed a significant prevalence ($P \leq 0.05$) of eyes symptoms in wheat flour mill workers compared with control subjects. Moreover , it was observed that there was significant increase ($p \leq 0.05$) in percentage of skin symptoms of wheat flour mill workers compared with control group.
The study illustrated that the concentration of sCD8 and sCD86 were significant elevated ($p \leq 0.05$) in total number of wheat flour mill workers compared with control group. The results also illustrated that positive correlation between concentrations of sCD8 and sCD86 with period of work of wheat flour mill workers.

1. INTRODUCTION:

Flour dust composed of many antigens or allergic proteins. The antigens involved are bacteria, endotoxins or lipopolysaccharide (LPS), fungal spores and insects (Liebers *et al.*,2006). Many authors Talini *et_al.*,2002; Das & Jha , 2009; Tawde *et al.*,2015 and Melo *et al.*,2016 reported that wheat flour dust responsible for respiratory symptoms, sensitization ,allergic rhinitis, occupational asthma and conjunctivitis. Wheat flour dust contains high molecular weight (HMW) antigens which act through an IgE-mediated (Type I) immunological pathway to cause contact dermatitis through a Type IV (cell-mediated) mechanism (Palosuo *et al.*,2003).
In addition, Ross *et al.*, (1997) ; Tatham & Shewry, (2008) illustrated that various allergens find in wheat flour that cause allergic asthma in flour millers. Moreover, Kollop-Sarda *et al.*, (1994) found that inhalation of flour dust in work environment stimulate immune responses when inhaled conditions. T cells have a crucial role in the induction of airway inflammation in asthmatic patients (Hacievliyagil *et al.*, 2013). Varney *et al.*, (1992) stated that T lymphocytes, mast cells, eosinophils, basophils and neutrophils are local accumulation in allergic inflammation. Mapp & Boschetto, (2003) suggested that not only CD4 T cells but also CD8 T cells were essential to the development of allergic inflammation. Saetta et al. (1998) that found increment number of $CD8^+$ T-lymphocytes in chronic airflow limitation.
Moreover, A soluble form of CD86 might play an important role in immune regulation by binding with the CD28 molecules, thus interfering with the binding of CD28 or/and cytotoxic T-lymphocyte–associated allergen (Chen and Shi,2006). Levels of serum sCD86 were significantly higher in acute asthmatics when compared to stable asthmatics and controls (Walker *et al.* 1991).
Therefore, This study was conducted to evaluate prevalence of eyes , skin symptoms and concentrations of sCD8 and sCD86 and relationships with duration of employment and in wheat flour mill workers in Najaf city in Iraq.

2. MATERIALS AND METHOD:

2.1. Study population:

This study was conducted in three major flour milling industries in AL-Najaf city. Those flour mill workers worked for at least 8–10 hours a day for 6 days per week. The study included determine of eyes and skin symptoms using questionnaire in 80 male non-smoking wheat flour mill workers aged between 18-48 years (mean age 34.01±8.17) years with a mean duration of exposure (8.7±4.23) years and 70 non-smoking unexposed healthy subjects of the same age, and body mass index group served as control (Table 1). Data was collected through direct interview in the workplace using prepared questionnaire. It contained items related to personal and family history of allergy, nose, eyes and skin symptoms and duration of employment.
The current study also investigated the concentrations of soluble CD86 and soluble CD8 in serum samples from for 48 (out of 80 workers) of non-smoking wheat flour mill workers , in addition 37 (out of 70 subjects) healthy subjects volunteers of the same age and body mass index as control group. Flour mill workers were divided into three subgroups depending on the job categories (sweepers [high exposure group], packers [intermediate exposure group] and millers [low exposure group]).

2.3. BMI (Body Mass Index) :

The weights and heights of the workers and controls were measured using a well calibrated scale. The weight was recorded in kilogram (Kg) and the height in centimeter. The body mass index (BMI) was calculated according to the following equation (Eknoyan, 2008)

$$BMI\ (Kg/m^2) = Weight\ (Kg)/\ (Height\ (m))^2$$

2.4. Blood samples:

Five milliliters of venous blood samples were collected from each worker and control and placed in serum tubes for evaluation concentrations of sCD68 and CD8 , these tubes centrifuged at 3000 rpm for 5 minutes to separate the serum which kept in new tubes, then put serum in epindroff tubes and kept at deep freeze (-20 C°) until used by (ELISA).

2.5.Measurement concentrations of sCD86 and CD8:

Human Soluble Cluster of Differentiation 86 (CD86) ELISA Kit and Human Cluster of Differentiation 8 (CD8) ELISA Kit were determined as declared manufacture company (US Biological, U.S.A) that used technique of the quantitative sandwich enzyme immunoassay.

Statistical analysis:

The data were statistically analyzed by using SPSS (statistical package for social sciences). The independent sample t-test, ANOVA (analysis of variance), chi square test, pearson correlation coefficient were used for in this study. All values were expressed as mean ± Standard Error of Mean. P-value less than 0.05 and 0.01 were considered statistically significant (Daniel, 2010).

3. RESULTS:

The physical parameters such as age and Body mass index (BMI) of both the control and wheat flour mill workers showed no significant difference (Table 1).

The study showed a significantly higher ($p \leq 0.05$) prevalence of eyes symptoms in wheat flour mill workers compared with control subjects (Table 2). In addition, the study indicated that red eyes (55%) and itchy eyes (37.5%) were the most common types of eyes symptoms in employees of mills (Table 2).

Moreover , it was observed that there was significant increase in percentage of skin symptoms of wheat flour mill workers compared with control group (Table 3). Furthermore, The results recorded that redness of skin (37.5%) and itchy skin (25%) were a higher percentage of skin symptoms in wheat flour mill workers (Table 3). However , the statistical analysis revealed that dry skin (15%) and skin (10%) were the lowest percentage of skin symptoms in mills workers (Table 3).

The study illustrated that the concentration of sCD8 and sCD86 were significant elevated ($p \leq 0.05$) in total number of wheat flour mill workers compared with control group (Table 4).

The results indicated that there was a significant increment ($p \leq 0.05$) in concentration of sCD8 sweepers and packers compared with the control group (Table 5). Furthermore, the results exhibited significant higher ($p \leq 0.05$) level of sCD8 in packers compared with sweepers and millers (Table 4).

Furthermore ,the study showed that there was a significant elevation ($p \leq 0.05$) in concentration of sCD86 in different job categories (sweepers , packers and millers) when compared with the control group (Table 5).

The results indicated that positive correlation between concentration of sCD8 and period for total number of wheat flour mill workers (Figure 1). Also, It can be clearly seen that highly significant positive correlation ($P<0.05$) between concentration of sCD86 and period for total number of wheat flour mill workers (Figures 2).

Moreover, the results demonstrated that positive correlation between concentrations of sCD8 and sCD86 with period of work of wheat flour mill workers (Figures 1 and 2).

Table (1) Physical characteristics of wheat flour mill workers and control subjects.

Parameters	Control	wheat flour mill workers	Sign. level
	Mean± SEM	Mean± SEM	
Age (years)	34.8±8.13	34.01±8.17	NS
BMI ($Kg/m^{2)}$	22.36±3.34	22.43±3.48	NS

Notes: SEM = Standard Error of the Mean . NS = No significant .

Table (2) The prevalence of eyes symptoms of wheat flour mill workers and control subjects.

Eyes symptoms	Wheat flour-exposed workers (n= 80)		Control (n=80)		p value
	Yes	No	Yes	No	
Red eyes	44 [a] (55%)	36 (45%)	6 (7.5%)	74 (92.5%)	<0. 01
Itchy eyes	30 [a] (37.5%)	50 (62.5%)	4 (5%)	76 (95%)	<0. 01
Conjunctivitis	22 [a] (27.5%)	58 (72.5%)	3 (3.8%)	77 (96.2%)	<0. 01
Watery eyes	14 [a] (17.5%)	66 (82.5%)	2 (2.5%)	78 (97.5%)	<0. 01

(a) = indicate statistically significant ($p \leq 0.01$) as compared with control.

Table(3) The prevalence of skin symptoms of wheat flour mill workers on the basis of job category and control grou

Skin symptoms	Wheat flour-exposed workers (n= 80)		Control (n=80)		p value
	Yes	No	Yes	No	
Redness of skin	30 [a] (37.5%)	50 (62.5%)	4 (5%)	76 (95%)	<0. 01
Itchy skin	20 [a] (25%)	60 (75%)	3 (3.8%)	77 (96.2%)	<0. 01
Dry skin	12 [a] (15%)	68 (85%)	2 (2.5%)	78 (97.5%)	<0. 01
Skin rash	8 [a] (10%)	72 (90%)	1 (1.3%)	79 (98.7%)	<0. 01

([a]) = indicate statistically significant ($p \leq 0.01$) as compared with control.

Table (4) Serum concentrations of sCD8 and sCD86 of wheat flour mill workers and control subjects.

Parameter	wheat flour mill workers (n=48)	Control (n=37)	P value
	Mean±SEM	Mean±SEM	
sCD-8 ng/ml	11.424[a]±0.48	8.82778±0.394	<0. 01
sCD-86 ng/ml	2.10781[a]±0.107	1.57335±0.05	<0. 01

([a]) = indicate statistically significant ($p \leq 0.01$) as compared with control.

Table (5) Serum concentration of sCD8 and sCD86 of wheat flour mill workers on the basis of job category and control group.

Parameters	Wheat flour-exposed workers (n= 48)			control (n=37)
	sweeper (n=16)	packer (n=16)	Miller (n=16)	
	Mean± SEM	Mean± SEM	Mean± SEM	Mean± SEM
sCD-8 ng/ml	10.77[a]±0.409	14.33[ab]±0.821	9.17[c]±0.689	8.82778±0.39
sCD-86 ng/ml	2.156[a]±0.177	2.19[a]±0.249	1.97[a]±0.113	1.57335±0.05

([a]) = indicate statistically significant ($p \leq 0.01$) as compared with control.
([b]) = indicate statistically significant ($p \leq 0.01$) as compared with sweeper.
([c]) = indicate statistically significant ($p \leq 0.01$) as compared with packer.

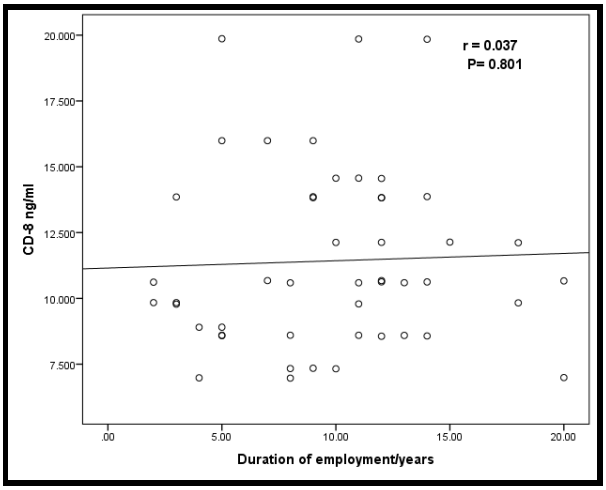

Figure (1) Correlation between serum concentration of CD8 and period of work of wheat flour mill workers.

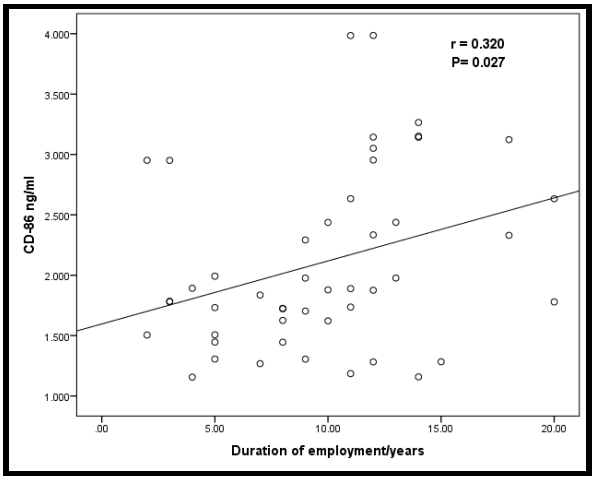

Figure (2) Correlation between the serum concentration of CD86 and period of work of wheat flour mill workers.

4. DISCUSSION:

The study found that eyes symptoms were significantly higher ($P \leq 0.05$) in wheat flour mill workers compared with control subjects (Table 2). This finding agreed with Ajeel & Al-Yassen, (2007) found 41.6 % of the workers in flour mills suffered from eye symptoms. In addition, This result agreed with the results obtained by Meo & Al-Drees, (2005) flour dust contains sensitive and dangerous substances induce respiratory, nasal and eye symptoms. The prevalence of eyes symptoms in flour mill workers might be caused by exposed workers at flour mill to high levels of flour dust during their work led to occupational airway disease and prevalence eyes symptoms.

Furthermore, the results indicated that there was significantly higher ($P \leq 0.05$) distribution of skin symptoms in wheat flour mill workers comparison with control subjects (Table 3). This result agreed with those reported by Fakhri (1992) who found higher prevalence of skin symptoms in flour mill workers due to long-term exposure to wheat flour. In addition, Steiner *et al*., (2011) ; Arrandale *et al.,* (2013) illustrated that various allergens find in wheat flour that cause hypersensitivity reactions in bakers and flour millers. This finding probably due to continuous contact with wheat flour includes different allergic proteins such as albumins , globulins, gliadins and glutens cause skin irritant that lead to allergic reactions.

In addition, The study elucidated that there was significant increment ($p \leq 0.001$) in serum levels of sCD8 and sCD86 in wheat flour mill workers compared with control group (Table 4). This finding is similar to results of other study by Gripenback *et al.*,(2003) found that wheat-flour exposure lead to increment in total leukocytes count and lymphocytes count because of immune response. This result might be explained by that flour mill workers were facing high concentrations of wheat flour particles in the workplace for eight to ten hours a day due to stimulate immune system to produce T cells. Kolopp-Sarda *et al.*,(1995) found exposure to wheat flour dust causes airways inflammation lead to recruitment of inflammatory cells in the lung. Lorenzo *et al.,* (1995) found exposure to allergen results significant increment in serum soluble molecules including sCD4 and sCD8 because of allergic reactions and inflammation.

Moreover, the results demonstrated that positive correlation between concentrations of sCD8 and sCD86 with period of work of wheat flour mill workers (Figures 1 and 2). These results might be attributed that inhalation of flour particles stimulate immune responses by inducing co-stimulatory signal also known as CD86. Sharpe and Freeman (2002) showed that the co-stimulatory ligands CD80 and CD86 play a crucial role in the initiation and maintenance of an immune response. Similar results obtained by Baskurt *et al.,* (1990) who found that positive correlation between concentration of sCD8 and long period of exposure to high concentrations of flour dust. Long-term employment in flour mills may lead to acute or chronic respiratory disease (Yach *et al.,*1985). Hilt *et al.*,(2002) reported exposure to air pollution stimulated production $CD8^+$ T cells lymphocytes. These results concluded that T cells lymphocytes referred to an inflammatory response. Also, Saetta *et al.* (1998) found increment number of $CD8^+$ T-lymphocytes in chronic airflow limitation. Eman *et al* (2003) illustrated that sCD86 use marker of chronic inflammation of the airways. Furthermore , George *et al.*,(2001) demonstrated that inhalation mice to grain dust with endotoxin for period eight weeks (four hours/day , five days/week) were associated with increment of $CD8^+$ T-lymphocytes because of airways hypersensitivity. Kollop-Sarda *et al.,* (1994) found that inhalation of flour dust in work environment stimulate immune responses. Walker *et al.* (1991) found increment concentration of serum sCD86 in acute asthmatics when compared with stable asthmatics. In addition, Dockery *et al.,* (1993) showed that continuous exposure to flour dust cause allergic response and inflammatory reaction.

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