

## Exposure to Respirable Flour Dust and Gliadin in Wheat Flour Mills

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**Abstract: Exposure to Respirable Flour Dust and Gliadin in Wheat Flour Mills: Iraj KHODADADI, et al. Department of Biochemistry and Nutrition, Faculty of Medicine, Hamadan University of Medical Sciences, Iran—Objectives:** The aim of this study was to determine the concentrations of respirable flour dusts and gliadin as well as gliadin-specific serum antibodies in exposed workers of Hamadan wheat flour mill factories. **Methods:** Blood samples from 95 exposed workers and 80 air samples from flour packing, husk packing, flour production and wheat unloading areas were collected. Respirable flour dust density was measured by a gravimetric method, and dust gliadin concentration as well as serum antibodies were determined by enzyme-linked immunosorbent assays. **Results:** The Time-weighted average (TWA) flour dust density was higher in all factories (1.64–4.68 mg/m<sup>3</sup>) compared with the threshold limit value (0.5 mg/m<sup>3</sup>) of ACGIH and showed a positive correlation with the gliadin concentration ( $p < 0.05$ ). Additionally, the respirable dust and gliadin concentrations were significantly higher at flour packing workstations compare with the other work areas. Moreover, the mean serum gliadin-specific IgA and IgG and total IgE antibodies were remarkably higher in exposed mill workers compared with the controls ( $p < 0.05$ ). **Conclusion:** We clearly demonstrated that workers in Hamadan flour mills are in exposed to a hazardous level of respirable flour dust, receiving the highest level of dust and gliadin in flour packing areas. Furthermore, dust-exposed workers showed upper levels of serum antibodies indicating exposure to higher amounts of

allergens than controls.

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**Key words:** Antibodies, Flour, Gliadin, Occupational Exposure, Respiration

It is clear that both genetic and environmental factors such as exposure to respirable dusts are important determinants of occupational disease<sup>1–4</sup>, and asthma arising from workplace exposure to cereal flour (bakers' asthma) is one of the commonest types of occupational asthma<sup>2,3,5</sup>. A threshold limit value of 0.5 mg/m<sup>3</sup> of flour dust was proposed in 2009 by the American Conference of Governmental Industrial Hygienists (ACGIH), and this is now being considered by the Iranian Technical Committee of Occupational Health as the occupational exposure level (OEL) in breathing zones for workers in flour mills<sup>6</sup>. Previous studies have shown that the prevalences of respiratory and allergic symptoms are higher in bakery workers compare with matched control subjects in some Iranian cities<sup>7–9</sup>; however, there is a limited information available on the flour dust concentration and/or wheat allergens in flour-related workplaces. The only available data on the respirable and total flour dust density in Iranian mill factories (Yasuj, Iran) showed a significantly high concentration of flour dusts in workplaces, indicating exposure of workers to hazardous levels of dusts<sup>10</sup>. Since a high prevalence of asthma and work-related respiratory symptoms (12–45%) was reported in bakers<sup>7,8</sup> in western Iran and nearly 30% of bakers had complaints about respiratory dysfunction according to a local Social Security Organization report, determination of the flour dust concentration at wheat flour-related workstations became a research priority in Hamadan Province.

Multiple allergens exist in the protein fraction of wheat flour that are responsible for respiratory dysfunctions and

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baker's asthma<sup>5, 11</sup>). Proteomic studies have shown that gliadin and glutenin account for a high proportion (80%) of the wheat proteins<sup>12-14</sup>. Gliadin and glutenin have also been found effectively implicated in wheat flour-related allergic diseases<sup>13, 15</sup>. Accordingly, immunological responses to flour exposure have been reported in bakers and mill workers based on the elevation of serum IgE, IgG and IgA antibodies<sup>16-18</sup>.

The aim of the present study was to determine the respirable flour dust density and the concentration of gliadin in breathing air at different workplaces in wheat flour mills (Hamadan Province, Iran). Serum total IgE as well as gliadin-specific IgG and IgA were also determined in flour dust-exposed workers.

## Subjects and Methods

### Materials

Air samples were collected with 224-PCXR4 Universal individual sampling pumps (SKC Inc., Eighty four, PA, USA) with a flow rate of 2.2 l/min. PTFE filters with a 25 mm diameter were obtained from Schuell GmbH (Dassed, Germany), and Bahar Afshan Ltd. (Tehran, Iran) provided 10-mm sampling cyclones and PVC pads. The ELISA kit for determination of gliadin in air samples (flour dust) was purchased from Tepnel Ltd. (Stamford, CT, USA), whereas Orgentec Diagnostika GmbH (Mainz, Germany) supplied ELISA kits for determination of gliadin-specific IgG and IgA antibodies in serum and the total IgE ELISA kit was provided by Monobind Inc. (Lake Forest, CA, USA).

### Study population

Ninety-five out of 123 workers (75%) from flour dust contaminated areas of 8 wheat flour mills, named A to H, were enrolled in this cross-sectional descriptive study. Additionally, 95 unexposed office workers were studied a control group, and a total of 190 blood samples were collected from test and control subjects. Smokers and workers with less than one year of work experience and subjects with previous lung and/or immunodeficiency disease history were excluded from the study. Workplaces including flour packing, husk packing, flour production and wheat unloading zones were considered as the most contaminated workstations in all factories, and 64 air samples were collected from these workplaces. Since different technologies and production equipment were being used in the different factories, the aforementioned workstations were not completely separated in some cases, and therefore, some workplaces shared common breathing air. However, in the process of collecting 8 air samples from each factory, three independent air samples were collected from each isolated workstation. Sixteen blank air samples were also collected (1 for every 4 test samples) in accordance with the National Institute for Occupational Safety and Health (NIOSH) methods<sup>19</sup>. Written informed

consent for participation was obtained, and the project was approved by the Research Ethics Committee of Hamadan University of Medical Sciences (Iran).

### Determination of respirable dust density

Breathing air samples from different workstations were collected in a 8 h period (one working shift) using a 224-PCXR4 individual sampler pump with a Higgins-Dewell (HD) cyclone attached to the breathing zone of workers according to the NIOSH protocols for aerosol sampling<sup>19</sup>. Briefly, the aerosols were collected on polytetrafluoroethylene (PTFE) membrane laminated filters. To determine particulate mass concentrations, PTFE filters that had been conditioned in desiccators for 24 h were weighed before and after sampling using a microbalance, and the time-weight average (TWA) of respirable dusts in air samples was then calculated<sup>19</sup>. During distribution to the flour mills, transport and storage, the filters were kept dry in closed cassettes at ambient temperature<sup>20</sup>.

### Determination of the gliadin concentration in respirable dusts

Flour dusts were extracted from PTFE filters by immersing the filters in a 0.15-M solution of phosphate buffered saline (PBS) on a shaker for 15 min and centrifuging them at 2,000 g for 2 min. The amount of gliadin in the supernatant was then determined using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions as previously described<sup>19, 20</sup>.

### Determination of serum antibodies

Serum anti-gliadin-specific IgA and IgG antibodies as well as serum total IgE were measured using an ELISA kit according to the manufacturer's instructions.

### Statistical analysis

The SPSS 14 and Curve Expert 1.3 softwares were used for statistical analysis of data. Results are presented as mean  $\pm$  SD, and the independent samples *t*-test was used to compare mean differences. Moreover, one-way ANOVA followed by post hoc, Tukey's and Dunnett's tests was used to analyze differences between groups, and regression analysis was used to determine correlation between variables.

## Results

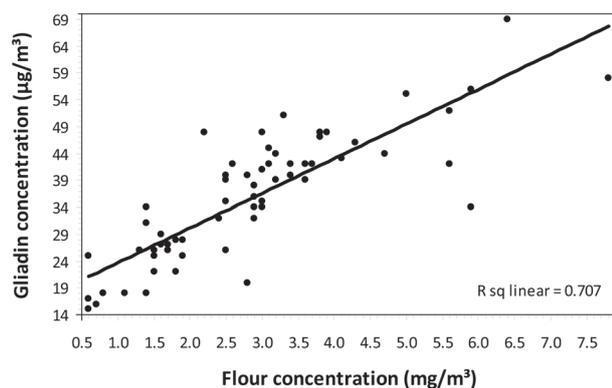
### Determination of respirable dust density

The density of respirable flour dusts was measured in air samples collected from flour mill factories. The density of respirable dusts was in the range of 0.55 to 7.77 mg/m<sup>3</sup>, and the mean density of respirable dusts was 2.79  $\pm$  1.54 mg/m<sup>3</sup>. The highest mean density of flour dusts was 4.68 mg/m<sup>3</sup> (Factory A), whereas the lowest dust density in air samples was 1.64 mg/m<sup>3</sup> (Factory B; Table 1). One-

**Table 1.** Respirable flour dust density in air samples collected from different wheat flour mills

Wheat flour factories	n	Mean ± SD	
		Density of respirable dusts (mg/m <sup>3</sup> )	Gliadin concentration (µg/m <sup>3</sup> )
Factory A	8	4.68 ± 1.88	50.10 ± 11.00
Factory B	8	1.64 ± 1.28	24.60 ± 10.00
Factory C	8	3.39 ± 1.16	42.70 ± 5.57
Factory D	8	3.74 ± 1.24	42.00 ± 9.30
Factory E	8	1.56 ± 0.63	26.00 ± 4.90
Factory F	8	3.00 ± 1.43	37.00 ± 9.90
Factory G	8	1.80 ± 0.75	27.37 ± 6.90
Factory H	8	2.49 ± 0.39	32.37 ± 7.85

way ANOVA also showed a significant difference in mean density of respirable dusts between different flour mill factories, with the density being significantly higher in Factory A compared with the other factories ( $p < 0.05$ ).



**Fig. 1.** Correlation of gliadin concentration and density of flour dusts in breathing air samples. Breathing air samples were collected from wheat flour mills. The density of respirable flour dusts and gliadin concentration in dust particles were determined, and regression analysis was performed ( $R^2 = 0.708$ ,  $p < 0.05$ ).

*Determination of gliadin concentration in respirable particles*

The gliadin concentration in flour particles ranged from 15 to 69 µg/m<sup>3</sup> with a mean density of 35.27 ± 11.81 µg/m<sup>3</sup> in workplace air from wheat flour mills in Hamadan Province. The maximum concentration of gliadin was 50.1 µg/m<sup>3</sup> (Factory A), while the minimum concentration of gliadin (24.6 µg/m<sup>3</sup>) was observed in respirable dust samples collected from Factory B (Table 1). In addition, one-way ANOVA showed a considerable difference in gliadin concentration between factories ( $p < 0.05$ ). Moreover, regression analysis demonstrated (Fig. 1) a strong direct correlation between respirable particle density in air samples and the concentration of gliadin ( $R^2 = 0.708$ ,  $p < 0.05$ ).

*Respirable dust density and gliadin concentration at different workstations*

Data analysis showed that respirable air (breathing zone) at different workstations contained significantly different concentrations of respirable flour dusts ( $p < 0.05$ ). While the maximum respirable dust concentration (3.5 mg/m<sup>3</sup>) was detected in flour packing areas (Table 2), flour production zones showed the lowest particle density (1.72 mg/m<sup>3</sup>). In the same way, a significant difference was observed in the mean gliadin concentrations of different workstations ( $p < 0.05$ ). The mean gliadin concentration

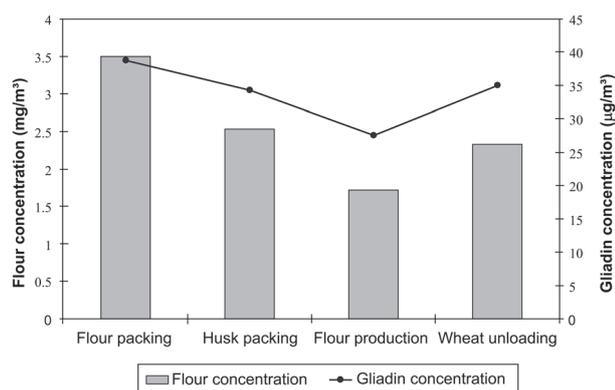
**Table 2.** Respirable flour dust density and mean gliadin concentration in air samples collected from different workstations at wheat flour mills

Workstations	n	Mean ± SD	
		Density of respirable dusts (mg/m <sup>3</sup> )	Gliadin concentration (µg/m <sup>3</sup> )
Flour packing	26	3.50 ± 1.80	38.7 ± 12.38
Husk packing	19	2.53 ± 1.18	34.3 ± 11.50
Flour production	9	1.72 ± 0.92	27.5 ± 8.40
Wheat unloading	10	2.33 ± 1.00	35.0 ± 11.22

was remarkably higher in flour packing areas ( $38.7 \mu\text{g}/\text{m}^3$ ), whereas the minimum mean concentration ( $27.5 \mu\text{g}/\text{m}^3$ ) was measured in air samples collected from flour production zones (Table 2). Moreover, regression analysis indicated a direct correlation between the respirable dust concentration and mean gliadin concentration in different workstations (Fig. 2).

#### Determination of serum antibodies

The independent sample *t*-test showed that the mean concentration of both serum gliadin-specific IgG and IgA antibodies as well as serum total IgE significantly differed in the control and test groups ( $p < 0.05$ ), and all 3 types of antibodies were markedly higher in the test group compared with the control (Table 3). Additionally, there was a great increase in mean total serum antibodies (sum of gliadin-specific IgG and IgA and serum total IgE antibodies) in test subjects compared with the controls ( $203.9 \text{ IU/ml}$  versus  $76.6 \text{ IU/ml}$ ,  $p < 0.001$ ).



**Fig. 2.** Gliadin concentration (line) accompanying the changes in the density of flour dusts (columns) at different workstations. Breathing air samples from different workstations were collected, the density of respirable flour dusts was determined and the gliadin concentration in dust particles was measured. One-way ANOVA followed by a post hoc test was used to analyze differences between groups, and Curve Expert 1.3 software was used to determine accompanying changes in variables.

## Discussion

Workplace air pollution is one of the major causes of diseases, and inhalation of organic or inorganic particles may result in a variety of respiratory dysfunctions and allergic reactions<sup>2,3</sup>. Wheat flour mill workers are exposed to flour dust and gliadin, the major wheat allergen, for 8–10 h per day<sup>16,17</sup> and therefore are prone to exhibit immunologic responses and allergic sensitization<sup>21,22</sup>.

Hamadan Province is one of the most ancient parts of Iran and Iranian civilization. The city of Hamadan, located in western Iran, is a partially developed city with a mid-economic state, semiarid traditional agriculture and semitransitional industries. The wheat flour mills in Hamadan Province that were enrolled in this study are differentially equipped with up-to-date technologies and exhibit different levels of flour dust exposure. The results of this study showed that the respirable dust density was significantly higher than  $0.5 \text{ mg}/\text{m}^3$  in all Hamadan flour mills<sup>6</sup>. This finding is in line with the only available report indicating exposure of flour mill workers to a hazardous level of inhalable and respirable flour dusts in Yasuj, which is in southwest Iran<sup>10</sup>. It should be noted that the threshold limit value (TLV) of  $0.5 \text{ mg}/\text{m}^3$  was proposed for inhalable dusts by the ACGIH and that a TLV for respirable dusts would be much lower than  $0.5 \text{ mg}/\text{m}^3$ . Therefore, respirable dust levels as measured in flour mills were tremendously higher than the actual TLV for respirable dusts. These observations suggest the requirement of careful and continuous monitoring of occupational exposure to flour dusts and consideration of a proper occupational health in flour mill workplaces. Statistical analysis also showed a significant difference in respirable dust density between different flour mills ( $p < 0.05$ ). In accordance with this observation, it is supposed that lesser flour dust is generated in modern factories, whereas a higher concentration of dusts was detected in older traditional flour mills.

It has been previously reported that wheat flour is an organic fine particle with an array of allergenic and antigenic proteins<sup>6,14,22</sup>, with the gliadin being considered as the main allergen<sup>16,17</sup>. Accordingly, the severity of sensitization disease related to flour dust and flour exposure is dependent on the type of these proteins<sup>11,23</sup>.

**Table 3.** Mean concentration of serum gliadin-specific IgA and IgG as well as total IgE in flour dust-exposed and control workers

Serum antibodies	n	Mean $\pm$ SD	
		Control subjects (IU/ml)	Exposed workers (IU/ml)
Anti-gliadin IgA	95, 95	$2.79 \pm 3.49$	$9.03 \pm 12.01$
Anti-gliadin IgG	95, 95	$6.29 \pm 10.04$	$48.06 \pm 47.01$
Total IgE	95, 95	$67.49 \pm 81.92$	$146.26 \pm 132.46$

Here, regression analysis showed that there is a positive correlation between respirable dust density and gliadin content of dust particles in air samples; that is, the gliadin concentration increases as the dust density increases ( $R^2=0.708$ ,  $p<0.001$ ). This finding is confirmed by the previous reports indicating an exposure response relation of wheat gliadin and respiratory symptoms<sup>24-27</sup>). These observations also suggest the need for more efficient local exhaust ventilation systems in the aforementioned workstations and personal protective equipment such as air purifying half masks with a proper dust cartridge, which efficiently reduce respiration of wheat allergens<sup>28</sup>).

Moreover, our observations confirmed that workers at flour packing workstations are exposed to an appreciably higher level of respirable dusts than others ( $p<0.05$ ). This finding together with the significantly higher gliadin concentration in manual flour packing areas ( $p<0.05$ ) indicates that workers in flour packing zones are receiving the highest level of dust and gliadin compared with the other workers in flour mills. Since the exposure intensity related response of lung function to respirable dusts has previously been reported<sup>24, 25, 27, 29</sup>), it is clear that workers at flour packing workstations are more prone to develop respiratory dysfunctions and allergic responses to wheat allergens. Congruent with the only previously published report in Iran<sup>10</sup>), this study confirms that the more manual operations in the mills, the more emission of flour dusts in workplace air. Therefore, workers such as packers, sweepers and sift operators who are directly exposed to flour inhale more flour dusts than others.

The increment of total IgE or gliadin-specific IgG and IgA antibodies has previously been shown in bakery and flour mill workers<sup>17, 24, 26, 27, 30</sup>). Similarly, we showed that gliadin-specific IgG and IgA antibodies as well as serum total IgE are markedly higher in flour mill workers than unexposed control subjects ( $p<0.001$ ). In addition, a great increase in mean total serum antibodies (sum of gliadin-specific IgG and IgA and serum total IgE antibodies) was observed in test subjects compared with the controls, confirming induction of allergic responses in flour-exposed workers<sup>22, 31, 32</sup>). However, no correlation has been found between serum gliadin-specific antibodies and age or years of working at a given workstation. The lack of a response of serum antibodies, as observed here, can be explained by the frequent changing of workstations for mill workers, such as from a flour packing to wheat unloading area, and by the assigning of newer workers to work in dusty areas. Constantly working (for at least one year) at a given workstation rarely was seen, and frequent switching between workplaces was a routine procedure for employees in all mills. The change in working area, for example from a contaminated area to an area with lesser exposure or vice versa, could obviously bias the results. Besides, sometimes dishonesty is a major limitation when using questionnaires in cross-sectional studies in third

world countries. To avoid losing their jobs, workers were usually reluctant to give correct information, particularly information about the effectiveness of ventilation systems, availability of personal protective equipment, and their health problems. Though there were no apparent complaints of daily symptoms such as shortness of breath, coughing or itching in subjects during working hours, our observations indicate that all workers in Hamadan flour mills were overexposed to flour dusts and showed higher serum antibodies compared with the control group regardless of their honesty or deceitfulness. However, a significant correlation between contamination status and blood antibodies was not observed under the limitations and/or conditions used in this study. Collectively, it seems that drawing a conclusion concerning the correlation of serum gliadin-specific antibodies and age or years of working requires an age- and workstation-matched study. The flour dust concentration was up to 10-fold higher than the TLV in flour mills, therefore, to meet the minimum workplace standard requirements according to the National Occupational Exposure Limit (NOEL), effective countermeasures should be considered to improve work situations, including replacement of manual packing processes by fully automated procedures and use of local exhaust industrial ventilation instead of general dilution ventilation in all factories. Furthermore, use of up-to-date technology in traditional mills and utilization of proper housekeeping and cleanup procedures to provide additional protection are recommended.

## Conclusion

We clearly demonstrated that the density of respirable dusts in Hamadan flour mills is higher than the threshold limit value (TLV). A significant direct correlation was also found between respirable dust density and gliadin content of dust particles in air samples. The observations made here are of immense importance because they indicate that workers in Hamadan flour mills are exposed to a high level of dust and gliadin. Flour packing workstations had the highest dust density and gliadin concentration compared with the other workplaces. Furthermore, dust-exposed workers showed upper levels of serum antibodies indicating exposure to higher amounts of allergens compared with controls. Therefore, effective ventilation systems and proper housekeeping procedures are needed to reduce occupational exposure of workers to flour dusts in wheat flour mills.

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