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Title: Comparison of subjective symptoms associated with exposure to low levels of formaldehyde between students enrolled and not enrolled in a gross anatomy course

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Abstract

Objectives This study aimed to evaluate students' subjective symptoms associated with exposure to low levels of formaldehyde (FA) during a gross anatomy course and to survey how the risk of subjective symptoms was affected by exposure to FA.

Methods We conducted three questionnaire surveys of 125 students enrolled in an anatomy course (FA exposure group) and 124 students not enrolled in the course (FA nonexposure group) before, during, and 6 months after the course. The questionnaire included questions inquiring about subjective symptoms, sex, age, and allergies. We analyzed differences in the prevalence of subjective symptoms in distinct survey periods. Furthermore, we analyzed the relationship between the subjective

symptoms and exposure to FA after adjusting for allergy, sex, and age using multiple logistic regression analysis.

Results The prevalence of some of the ocular, nasal, and nonspecific symptoms in the FA exposure group was low before the course, increased during the course, and decreased 6 months after the course. A significant positive relationship was observed between exposure to FA and some symptoms after adjusting for allergy, sex, and age.

Conclusions We identified some concrete symptoms associated with exposure to FA. We suggest that the exposure to low levels of FA influences students' subjective symptoms.

Introduction

In Japan, medical students and lecturers are exposed to formaldehyde (FA) during gross anatomy dissection courses. There is cause for concern that this exposure level triggers FA-associated symptoms, including headache, fatigue, and irritation of the eyes, nose, and throat. Furthermore, exposure to FA during dissection classes may induce specific IgE, but not IgG, against FA-albumin [1]. Experiments on animals have also demonstrated that inhalation of FA has been shown to inhibit learning and memory performance in mice [2]. In addition, other animal experiments have demonstrated that even the inhalation of low levels (0.1 ppm) of FA affected oxidant stress in a tissue-specific manner [3].

To reduce exposure to FA during dissection classes, many Japanese universities have measured FA concentrations in gross anatomy

61 laboratories and performed laboratory repair work, which has led to

62 improvements of laboratory environment [4-8].

63 In our university, we have taken countermeasures to reduce exposure to

64 FA during dissection classes since 2006. We attempted to reduce the

65 indoor levels of FA in a gross anatomy dissection room using a stepped

66 intervention between 2006 and 2008 [9- 10]. Furthermore, large-scale

67 repair work was performed between January and March 2011. The repair

68 work comprised the installation of dissection tables equipped with local

69 ventilation systems and the renewal of the general ventilation system.

70 After these improvements, the indoor levels of FA decreased by nearly

71 90% compared with the levels observed in 2008. To assess the effects of

72 this repair work, we also conducted a questionnaire survey to inquire

73 about subjective symptoms before and after these large-scale repairs. After

the repair work, the number of individuals with subjective symptoms significantly decreased in 2011; however, not all symptoms disappeared [11]. There are very few studies that have assessed the health effects of exposure to post-repair FA levels on medical students and experimental animals with the exception of our previous studies [11- 12]. Therefore, it is necessary to continue measuring the indoor FA concentrations and devise further strategies aimed at reducing exposure to FA even in an environment of low FA levels within the exposure limits set by the Japan Society for Occupational Health (0.1ppm) [13].

In our previous study, we evaluated the prevalence of subjective symptoms in students who enrolled in a gross anatomy course after large-scale repair work at our university. Although the prevalence of many subjective symptoms increased during the course compared with the

prevalence before the course, the prevalence of many symptoms decreased 6 months after the end of the course [12]. However, the increased prevalence of many subjective symptoms during the course may be caused by factors other than exposure to FA such as seasonal allergy, sex, and age. In this study, to assess the specific effect of exposure to low levels of FA on students' subjective symptoms, we distributed questionnaires about 16 subjective symptoms and allergies to two groups of college students, enrolled or not in the anatomy course and compared trends between the groups. We also aimed to evaluate specific subjective symptoms associated with exposure to FA and whether the risk of subjective symptoms increased among students by exposure to FA.

Materials and Methods

Study population

The study population comprised 124 first-year medical students and 125 second-year medical students at Kurume University's School of Medicine. All second-year students were enrolled in afternoon gross anatomy classes between April and July 2013. None of the first-year students had previously enrolled in gross anatomy courses. The second-year students enrolled were designated 'FA exposure group', and the first-year students not enrolled were designated 'FA nonexposure group'.

Study design

The students completed self-administered questionnaires three separate times: April 2013 (immediately before the course), May 2013 (during the course), and January 2014 (6 months after completion of the course). The

questionnaire included questions on 16 subjective symptoms associated with eye, nose, and throat complaints, as well as unidentified complaints, and age, sex, and allergies diagnosed by a doctor. The responses to questions about the frequency of subjective symptoms were “never,” “sometimes,” or “often.” We examined the relationship between exposure to FA and subjective symptoms using multiple logistic regression analysis, and the symptoms identified were independently associated with exposure to FA during the course.

We simultaneously measured the indoor FA levels at 5 locations during a gross anatomy class on the same date the questionnaires were completed. We selected the FA levels in the dissection room in May 2013 as the representative FA level during the course, because those levels were measured during the thoracotomy procedure, and we expected the FA level

would be the highest during that procedure relative to all other classroom practices. For this purpose, we collected 10 L of air at a flow rate of 0.5 L/min at a height of 1.2 m above the floor using a portable pump (Gastec GSP-250FT) and determined the indoor FA levels using high-performance liquid chromatography (HPLC) [14].

Statistical analysis

Students who answered that they sometimes or often experienced each subjective symptom were classified as having those subjective symptoms. We calculated the percentage of students having each subjective symptom in relation to the total number of students enrolled and labeled this category “prevalence of subjective symptoms.” Differences in the prevalence of each subjective symptom in each survey period were

compared using Cochran’s Q test and McNemar's test. Multiple logistic regression analysis was performed considering the presence or absence of subjective symptoms as the dependent variable, and considering the exposure to FA, allergies diagnosed by a doctor (with or without allergy symptoms), sex, and age at baseline (continuous variable) as the independent variables in each survey period. The prevalence of each subjective symptom, sex and allergies were statistically compared between the FA exposure group and the FA nonexposure group using the chi-square test. Differences in age were statistically compared between the two groups using the unpaired *t*-test. All statistical analyses were performed using IBM SPSS Statistics software for Japan version 19. The statistical significance was two-tailed and set at $P < 0.05$ for all analyses.

Ethical considerations

This study was approved by the Research Ethics Committee of Kurume University. All participants were informed about the content and objectives of this study, and the response to the questionnaire was considered as consent to participate.

Results

We analyzed the answers of 123 second-year medical students and 114 first-year medical students from the questionnaires administered before, during, and 6 months after the course (the response rate was 98.4% for the second-year students and 93.9% for the first-year students).

Table 1 shows the characteristics of the study population in May 2013. The mean age differed significantly between the two groups. No

significant differences between sex and allergy history were observed between the two groups.

Figure 1 shows the prevalence of each subjective symptom before, during, and 6 months after the course in the FA exposure group. There were significant differences in the prevalence of subjective symptoms such as eye soreness, eyestrain, itchy eye, tearing, itching of nose, nasal obstruction, sore throat, fatigability, and listlessness among three survey times.

Figure 2 shows the prevalence of each subjective symptom before, during, and 6 months after the course in the FA nonexposure group. There were significant differences in the prevalence of subjective symptoms such as itching of nose, runny nose, sore throat, dry throat, fatigability, and listlessness among three survey times.

Table 2 shows the odds ratios (ORs) of subjective symptoms in the FA exposure group compared with the FA nonexposure group in each survey period. During the anatomy course, a significant positive relationship was observed between exposure to FA and tearing (OR 2.62; 95% confidence interval [CI] 1.36- 5.04), fatigability (OR 2.42; 95% CI 1.38- 4.26), eye soreness (OR 2.35; 95% CI 1.30- 4.27), listlessness (OR 2.09; 95% CI 1.19- 3.66), eyestrain (OR 1.82; 95% CI 1.07- 3.14), and itching of nose (OR 1.76; 95% CI 1.01- 3.06) after adjusting for the variables allergy, sex, and age. Before the course and 6 months after the course, no significant positive relationship was observed between exposure to FA and subjective symptoms, whereas there were significant negative relationship between exposure to FA and some subjective symptoms.

Table 3 shows the effects of allergy and sex (being female) on subjective symptoms using logistic regression analysis during the gross anatomy course. The symptoms significantly associated with allergy during the course were runny nose (OR 2.85; 95% CI 1.62- 5.03), itching of nose (OR 2.78; 95% CI 1.58- 4.90), and nasal obstruction (OR 2.30; 95% CI 1.31- 4.06). The symptoms significantly associated with being female during the course were dry eye (OR 2.43; 95%CI 1.30- 4.53), itchy eye (OR 2.42; 95%CI 1.29- 4.53), and fatigability (OR 2.06; 95%CI 1.10- 3.88).

The mean FA levels (standard deviation) across 5 locations in the dissection room during the thoracotomy procedure was 0.11 (0.02) ppm. The volume of the dissection room was 1,133 m^3 and the floor area was 357 m^2. There were 31 dissection tables in this room. The air exchange rate in this room was 16.8 per hour.

Discussion

We examined the specific effects of exposure to low levels of FA—, i.e., levels within the exposure limits set by the Japan Society for Occupational Health—, on students' subjective symptoms during an anatomy course by simultaneously comparing the health status of students enrolled and not enrolled in the course. In the FA exposure group, there were significant differences in the prevalence of eye soreness, eyestrain, itchy eye, tearing, itching of nose, nasal obstruction, sore throat, fatigability, and listlessness among three survey times. The prevalence of these seven symptoms except for itchy eye and nasal obstruction was low before the course, increased during the course, and decreased 6 months after the course. However, the prevalence of these symptoms in the FA nonexposure

group was low during the course or gradually increasing as time progressed. Therefore, these results suggest that exposure to low levels of FA during the course had an impact on ocular symptoms, nasal symptoms, and non-specific symptoms.

In the FA nonexposure group, the prevalence of some eye and nose complaints before the course was higher than that observed during the course. This could be due to seasonal factors such as pollen allergies and other allergies, as we showed previously [12]. However, in the FA exposure group, the prevalence of some symptoms during the course was higher than that observed before the course. This result suggests that exposure to FA influenced the symptoms more strongly than seasonal variables.

During the course, a significant positive relationship was observed between exposure to FA and some ocular, nasal, and non-specific symptoms after adjusting for allergy, sex, and age. There were no significant subjective symptoms that ORs were more than one before the course and 6 months after the course, whereas ORs of some subjective symptoms were less than one significantly before the course and 6 months after the course (Table 2). Therefore, it has been suggested that the exposure to low levels of FA greatly influences these symptoms. The results of the present study were in agreement with those of our previous study in which the prevalence of many symptoms had decreased 6 months after the course [12].

Some nasal symptoms were also significantly associated with allergies. In this respect, special care should be taken to avoid worsening the allergy

241 outcomes among individuals who have allergies. Several strategies can be
242 implemented to achieve this goal, including the reallocation of individuals
243 who have allergies to safety laboratory zones where FA concentrations are
244 lower, promotion of allergy therapies, and the provision of thorough
245 instructions on the use of personal protective equipment (PPE). In addition,
246 we showed that some symptoms were significantly associated with sex.
247 Female students tended to complain more about their symptoms compared
248 with male students, a result that was also similar to that of our previous
249 study [9, 15- 16]. Therefore, future efforts should focus on attempts to
250 minimize students' exposure to FA and promote safety education
251 particularly for students who have allergies and for female students.
252 To minimize students' exposure to FA, we recommend the use of PPE
253 such as gas masks and eye protectors. In this study, the percentage of

254 students using PPE was very low. Only four students (3.3%) wore half-
255 mask air-purifying respirators for FA (gas masks) or masks with activated
256 carbon filters. However, most students (92.7%) wore disposable non-
257 woven masks, which do not prevent exposure to FA (simple masks). In
258 addition, only four students (3.3%) wore eye protectors. The efficacy of
259 wearing PPE to prevent subjective symptoms has been addressed in a
260 previous study [17]. In our study, we analyzed the association between
261 wearing PPE for FA and the occurrence of subjective symptoms and no
262 significant association was found. One explanation for this result is that
263 we did not evaluate the efficacy of PPE in reducing subjective symptoms
264 because only a few students wore PPE for FA. Another reason is that
265 students having subjective symptoms might have worn PPE to suppress
266 these symptoms. Fifty-four (45.8%) of the 118 students who wore masks—,

267 including gas masks for FA, masks with activated carbon filter, or simple

268 masks—, and three (75.0%) of the four students who wore eye protectors

269 reported that they felt more comfortable wearing masks and eye protectors.

270 In addition, we believe that simple masks are efficient only in cases in

271 which FA concentrations are low. It is essential to wear suitable PPE and

272 encourage many students enrolled in the course to use gas masks and eye

273 protectors by allowing the students to try them on or borrow them.

274 The FA level of one laboratory site evaluated on the day of the

275 questionnaire survey was within the exposure limits set by the Japan

276 Society for Occupational Health (0.1 ppm) [13]. A previous study

277 reported that the olfactory threshold of FA was between 0.06 and 0.07

278 mg/m^3 (between 0.048 and 0.056 ppm), and many individuals tend to

279 experience irritative symptoms when the FA concentration exceeded this

threshold [5]. In the present study, the FA level exceeded the olfactory threshold of FA. Therefore students would have tended to experience irritative symptoms by FA.

The present study has two limitations. The first limitation is the effect of behaviors other than the anatomy practice on subjective symptoms. The activities at a university such as other practice, lecture or club and off-campus activities such as part-time jobs that influence students' stress responses in the survey may be different for each grade. The disturbances in the lifestyle by these activities might affect their subjective symptoms. However, we could not assess activities other than the anatomy practice with our questionnaire. Additionally, the stress of students may increase because students are medically knowledgeable about the harmful effects of FA. We should evaluate the stress levels caused by the anatomy practice

293 itself and other events by using a stress scale, together with our

294 questionnaire. By doing this, we can assess the psychological influence of

295 exposure to low levels of FA and the stress responses caused by various

296 factors.

297 The second limitation is the effect of other chemicals on subjective

298 symptoms during the anatomy practice. At our university, students dissect

299 cadavers fixed with 3.7% FA. After every class, students spray an

300 antimycotic agent on the surface of the body of the donor. The antimycotic

301 agent consists primarily of *N*, *N*-dimethyl-*N*'-phenyl-*N*'-sulfamide. Traces

302 of these chemical substances in agents that remain after previous courses

303 may have influenced the subjective symptoms. We estimated that the

304 effect of the antimycotic agent was little if any because students sprayed a

305 small amount of agent after each class. Although there was a possibility

306 that students had been exposed to chemical substances during other

307 practices, we confirmed that students were not exposed to chemicals

308 around the same time as the anatomy practice.

309 In this study, we aimed to assess specific effect of exposure to FA on

310 students' subjective symptoms during the anatomy course. Accordingly,

311 we identified some concrete symptoms such as ocular symptoms, nasal

312 symptoms, and non-specific symptoms associated with exposure to FA

313 after adjusting for allergy, sex, and age using multiple logistic regression

314 analysis. We will continue to monitor students' health status by using

315 these symptoms as an index. In addition, we intend to investigate the

316 effect of the exposure to low levels of FA on students' learning by using

317 objective performance indexes.

318

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Wantke F, Focke M, Hemmer W, Bracun R, Wolf-Abdolvahab S, Gotz M, et al. Exposure to formaldehyde and phenol during an anatomy

dissecting course: sensitizing potency of formaldehyde in medical students. Allergy. 2000;55:84-7.

2. Lu Z, Li C.M, Qiao Y, Yan Y, Yang X. Effect of inhaled formaldehyde on learning and memory of mice. Indoor Air. 2008;18:77-83.

3. Matsuoka T, Takaki A, Ohtaki H, Shioda S. Early changes to oxidative stress levels following exposure to formaldehyde in ICR mice. J Toxicol Sci. 2000;35:721-30.

4. Kunugita N, Nakashima T, Kikuta A, Kawamoto T, Arashidani K. Exposure to formaldehyde during an anatomy dissecting course. J UOEH. 2004;26:337-48 (in Japanese with English abstract).

5. Mizuki M, Tsuda T. Relationship between atopic factors and physical symptoms induced by gaseous formaldehyde exposure during an

anatomy dissection course. Japanese Journal of Allergology. 2001;50:21-8 (in Japanese with English abstract).

6. Ohmichi K, Matsuno Y, Komiyama M, Fukuda H, Todaka E, Ohta M, et al. Study of formaldehyde concentration during gross anatomy laboratory in Chiba University. Jpn J Clin Ecol. 2005;14:112-8 (in Japanese with English abstract).

7. Takayanagi M, Sakai M, Ishikawa Y, Murakami K, Kimura A, Kakuta S et al. Formaldehyde concentrations in the breathing zone of medical students during gross anatomy laboratory in Toho University. Kaibogaku Zasshi. 2007;82:45-51 (in Japanese with English abstract).

8. Tanaka K, Nishiyama K, Yaginuma H, Sasaki A, Maeda T, Kaneko S, et al. 2003. Formaldehyde exposure levels and exposure control measures

during and anatomy dissecting course. Kaibogaku Zasshi. 2003;78:43-51 (in Japanese with English abstract).

9. Mori M, Hara K, Ishitake T, Saga T, Yamaki K. Research on indoor levels of formaldehyde and subjective symptoms in medical students during a gross anatomy dissection course. Jpn J Clin Ecol. 2008;17:13-20 (in Japanese with English abstract).

10. Mori M, Hoshiko M, Hara K, Ishitake T, Saga T, Yamaki K. Reduction in indoor levels of formaldehyde in a gross anatomy dissection room by gradual intervention. Jpn J Clin Ecol. 2011;20:123-30 (in Japanese with English abstract).

11. Mori M, Hoshiko M, Hara K, Ishitake T, Saga T, Yamaki K. Effect of large-scale repair work on indoor formaldehyde levels and subjective

symptoms in medical students during a gross anatomy dissection course. Jpn J Hyg. 2012;67:501-7 (in Japanese with English abstract).

12. Mori M, Hoshiko M, Hara K, Saga T, Yamaki K, Ishitake T. Changes in subjective symptoms and allergy state among medical students exposed to low-level formaldehyde 6 months after completion of a gross anatomy dissection course. Environ Health Prev Med. 2013;18:386-93.

13. JSOH (Japanese Society of Occupational Health). Recommendation of occupational exposure limits (2015-2016). Tokyo: The Japan Society for Occupational Health. J Occup Health. 2015;57:394-417.

14. Mori M, Hara K, Miyakita T, Ishitake T. Improvement in indoor air quality and physical health of users in a newly built school building in a university. Jpn J Hyg. 2011;66:122-8 (in Japanese with English abstract).

15. Hojo S, Ishikawa S, Kumano H, Miyata M, Matsui T, Sakabe K. Subjective and objective characteristics of patients with multiple chemical sensitivity in Japan. Jpn J Clin Ecol. 2007;16:104-16 (in Japanese with English abstract).

16. Takigawa T. Indoor air pollution by chemical substances and sick house syndrome. JJOMT. 2006;54:193-9 (in Japanese with English abstract).

17. Iwasawa S, Tsuda Y, Uchiyama T, Maruyama Y, Kanda H, Miyauchi H, et al. Effectiveness of wearing protective devices (gas masks and eye protectors) against exposure to formaldehyde during an anatomy lab course. J Science of Labour. 2009;85:120-31 (in Japanese with English abstract).

Table 1. Characteristics of the study population in May 2013

*P <0.05; **P <0.01.

【Table can be found in another attached file.】

Table 2. Odd ratios of subjective symptoms in FA exposure group compared with FA nonexposure group in each survey period

*P <0.05; **P <0.01.

[a] The dependent variable was the presence or absence of each subjective symptom; independent variables were exposure to FA (grade), allergy, sex, and age.

[b] We defined the odds ratios of each subjective symptom in the FA nonexposure group as 1.

【Table can be found in another attached file.】

Table 3. Effect of allergy and sex (being female) on subjective symptoms using logistic regression analysis during a gross anatomy course

*P <0.05; **P <0.01.

[a] The dependent variable was the presence or absence of each subjective symptom; independent variables were exposure to FA (grade), allergy, sex, and age.

[b] We defined the odds ratios of each subjective symptom in the FA nonexposure group as 1.

【Table can be found in another attached file.】

Mihoko Mori, et al. (Fig. 1)

Fig. 1

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Mihoko Mori, et al. (Fig. 2)

Fig. 2

【Figure can be found in another attached file.】

Figure legends

Fig. 1 Prevalence of subjective symptoms before, during, and 6 months after a gross anatomy course in the formaldehyde (FA) exposure group. *Difference between the prevalence before the course or during the course and 6 months after the course, $P < 0.05$; ** difference between the prevalence before the course or during the course and 6 months after the course, $P < 0.01$.

Fig. 2 Prevalence of subjective symptoms before, during, and 6 months after gross anatomy course in the formaldehyde (FA) nonexposure group. *Difference between the prevalence before the course or during the course and 6 months after the course, $P < 0.05$; ** difference between the

443 prevalence before the course or during the course and 6 months after the

444 course, $P < 0.01$.