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Original article

Comparison of Overall Immunity Levels among Workers at Grape Orchard, Rose Greenhouse, and Open-Field Onion Farm

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ABSTRACT

Background: Occupational hazards in crop farms vary diversely based on different field operations as soil management, harvesting processes, pesticide, or fertilizer application. We aimed at evaluating the immunological status of crop farmers, as limited systematic investigations on immune alteration involved with crop farming have been reported yet.
Methods: Immunological parameters including plasma immunoglobulin level, major peripheral immune cells distribution, and level of cytokine production from activated T cell were conducted. Nineteen grape orchard, 48 onion open-field, and 21 rose greenhouse farmers were participated.
Results: Significantly low proportion of natural killer (NK) cell, a core cell for innate immunity, was revealed in the grape farmers (19.8 ± 3.3%) in comparison to the onion farmers (26.4 ± 3.1%) and the rose farmers (26.9 ± 2.5%), whereas cytotoxic T lymphocyte proportion was lower in the grape and the onion farmers than the rose farmers. The proportion of NKT cell, an immune cell implicated with allergic response, was significantly higher in the grape (2.3 ± 0.3%) and the onion (1.6 ± 0.8%) farmers compared with the rose farmers (1.0 ± 0.4%). A significantly decreased interferon-gamma:interleukin-13 ratio was observed from *ex vivo* stimulated peripheral blood mononuclear cells of grape farmers compared with the other two groups. The grape farmers revealed the lowest levels of plasma IgG1 and IgG4, and their plasma IgE level was not significantly different from that of the onion or the rose farmers.
Conclusion: Our finding suggests the high vulnerability of workplace-mediated allergic immunity in grape orchard farmers followed by open-field onion farmers and then the rose greenhouse farmers.

1. Introduction

With a global rise in population, higher demand for agricultural products has greatly influenced the agricultural system worldwide. Intensive farming practices such as extensive soil management, excess use of fertilizers or pesticides, and large-scale livestock confinements are widely used to increase productivity [1,2]. Although the agricultural sector is known as one of the most hazardous industries, it is estimated to have one of the greatest labor forces with over a billion people worldwide and employ about 450 million waged women and men workers [3]. Meanwhile, this huge workforce highly contributes to the rise in the global burden on occupational diseases and deaths [4].

Agricultural workers could be exposed to a composite mixture of different organic, inorganic, and microbial agents through inhalation or skin absorption. Livestock farm environments are

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Table 1
Demographic information on study subjects

Occupation	Location	Gender*	No. subjects	Age (year)†	Working duration (year)†	No. smokers (smoking year)‡	No. subjects with respiratory illness§
Onion farmers	Naju	Women	10	63.5 ± 10.2	24.2 ± 15.3	0	1
		Men	10	68.1 ± 9.6	49.8 ± 16.8	6 (36.0 ± 5.5)	1
Grape farmers	Damyang	Women	4	56.5 ± 3.5	15.3 ± 13.3	0	1
		Men	15	67.3 ± 7.1	26.4 ± 21.5	10 (25.5 ± 14.5)	2
Rose farmers	Gangjin	Women	11	54.4 ± 7.9	14.7 ± 9.0	0	1
		Men	10	59.3 ± 5.8	19.2 ± 9.4	8 (17.3 ± 9.7)	1

Data are expressed as mean ± SD.
* No significant difference in gender distribution among the farming groups.
† No significant differences in mean age and working duration among the farming groups within same gender.
‡ Significant difference ($p < 0.05$) between onion and rose farmers.
§ Diagnosed with chronic obstructive pulmonary disease, asthma, or allergic rhinitis at hospital in the past.

strongly contaminated with organic dust containing microbial contaminants and gaseous pollutants [5,6], whereas hazardous agents in crop farms vary widely. Field operations such as soil tillage, pesticide or fertilizer application, harvesting, and post-harvest processes account for diverse workplace exposure in different crop farms [7–9]. Various allergic responses such as asthma, farmers' lung disease, hay fever, hypersensitivity pneumonitis as well as interstitial lung disease, and cancer have been widely reported in workers at dairy farms, livestock confinements, greenhouse, and open-field farms [10], but the protective effect of endotoxin exposure from farming environment against allergic diseases has also been demonstrated to children grown up in farming households [11,12]. Nevertheless, workplace-mediated health effects may depend on exposure variability based on geographic location, work practices, and use of diverse workplace settings such as greenhouses, orchards, open fields, or animal confinement [13,14].

Among agricultural sectors, animal husbandry stands out as the most extensively studied area. Primarily, immune profiling of swine or poultry farmers has reported a predominant type-2 helper T cell (T_H2) response, which is suggestive of allergic response promotion in animal confinements [15,16]. Meanwhile, a study on the immune status of Thai orchid farmers following excess pesticide exposure reported an elevated serum IgE level and low B cell proportion, but no incidence of allergic conditions was noted in subjects [17]. According to the best of our knowledge, few studies on the work-mediated modulation of overall immune parameters have ever been conducted so far to crop farmers but no immunologic evaluation for grape orchard, onion open-field, or rose greenhouse workers.

Regarding organic dust exposure as a critical factor for the occurrence of occupational respiratory diseases in agricultural workers [5–7,9,10,12], studies on assessment of particulate matter (PM) concentration in crop farms including orchards and open-field farms have already reported higher PM exposure concentration than the determined threshold limits [7,18,19]. In addition, manifestation of various respiratory or dermal allergic reactions has been widely recognized in different crop farmers [20–22], whereas *in vitro* studies on farm dust exposure to alveolar macrophage and epithelial cells reported variable results of inflammation or oxidative stress induction [12,23,24]. Considering the differences in occupational exposure to various hazardous agents from planting, tillage, or harvest activities at grape orchard, onion open-field, or rose greenhouse farming, it could be assumed that these farmers could demonstrate differences in overall immune status. For this, the present study compared hematological variables, plasma immunoglobulin levels, proportion of major immune cells, and cytokine production from peripheral blood mononuclear cells (PBMCs) among those three crop farming worker groups.

2. Materials and methods

2.1. Study population and blood collection

A total of 20 onion, 19 grape, and 21 rose farmers from Jeolla-Namdo province, Korea, voluntarily participated in this study with their written consent. These farmers, who have involved solely with each crop farming, were participated. Ten milliliters of venous blood sample was collected between July to September in the year 2019 and 2020. Authorization for sample collection was provided by the Institutional Review Board of Daegu Catholic University (approval #CUIRB-2019-0004/-01). No significant difference was observed with the distribution of sex among the farming groups, and the mean age was not significantly different among the farming groups within same gender (Table 1). For hematological analysis, complete blood count and differential leukocyte count were determined by using Advia 2120 automatic blood analyzer (Siemens, Munich, Germany).

2.2. Plasma collection and PBMC isolation

Plasma was obtained by centrifugation of the blood sample, whereas PBMCs were isolated by Ficoll-Hypaque density-gradient centrifugation (Ficoll-Paque, GE healthcare Biosciences, Uppsala, Sweden). After counting and assessing viability, cells were suspended in complete Roswell Park Memorial Institute Medium (RPMI) media comprising RPMI 1640, 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 1 mM non-essential amino acid, 1% (w/v) sodium bicarbonate, 1% penicillin-streptomycin, and 50 mM 2-mercaptoethanol.

2.3. T cell stimulation and lymphocyte phenotyping

Isolated PBMCs (10^6/mL/well) were stimulated with 5 ng phorbol 12-myristate 13-acetate, 500 ng ionomycin, and 10 units of human recombinant interleukin (IL)-2 in 24-well plate for 72 hr at 37°C in a 5% CO_2 incubator. Cytokine production was measured from the culture supernatant. Phenotyping of lymphocyte subpopulation was done using Four-color flow cytometry (BD Accuri™ C6 Plus, San Jose, USA). Anti-CD3 FITC, anti-CD19 PE, and anti-CD56 PE (BD Pharmingen, San Jose, USA) were used to phenotype T cell, B cell, and natural killer (NK) cell populations. T cell phenotyping for helper, cytotoxic, and NKT cell was done with anti-CD4 PerCP, anti-CD8 PE, and anti-CD16 PerCP-Cy™5.5 (BD

Pharmingen, San Jose, USA). Each fluorescence conjugated isotypes control was used for subtracting the non-specific background binding of fluorescent antibodies.

2.4. Plasma immunoglobulin quantitation

Measurement of plasma IgG1, IgG4, and IgA was performed by sandwich ELISA as previously described [25,26]. Plasma IgE level was measured using Total IgE kit (IBL International, Hamburg, Germany) following the instructions provided.

2.5. Cytokine measurement

Commercially available ELISA sets for tumor necrosis factor-alpha (TNF-α), IL-4, interferon-gamma (IFN-γ; BD Biosciences, San Diego, USA), and IL-13 (R&D Systems, Minneapolis, USA) were used according to the manufacturer's instructions for measuring respective cytokine levels in the culture supernatant. For determining the balance between type-1 helper T cell (T_H1) and T_H2 reactivity, the ratio of IFN-γ:IL-13, representative cytokine produced from T_H1 and T_H2 cells, respectively [15,16], was calculated.

2.6. Statistical analysis

Sigma Plot 14.0 (Systat Software Inc., San Jose, USA) was used for statistical analyses. Using the Chi-square test, gender distribution and comparison of smoking percentage or prevalence of respiratory illness among the farming groups were analyzed. When data passed the normality test, one-way analysis of variance was used, whereas Kruskal-Wallis test by ranks was executed upon failing the normality test. Furthermore, when the significant difference between the groups existed, the Holm-Sidak method or Dunnet method was used as post-hoc analysis and further confirmed by Student *t* test or Mann-Whitney U test. Differences with $p < 0.05$ were considered significant.

3. Results

3.1. Epidemiological characteristics related with immunity level

Information on epidemiological factors, which may influence on overall immunity level related with respiratory illness, was collected. No significant differences were observed with the mean age and the mean duration of each crop farming among the farming groups within same gender (Table 1). Although smoking percentage of men farmers was not significantly different among onion, grape, and rose farmers (60, 67, and 80%, respectively), year of smoking was significantly longer to onion farmers than rose farmers. The number of subjects diagnosed at hospital with various respiratory illnesses, including chronic obstructive pulmonary disease, asthma, or allergic rhinitis, was also not significantly different among the farming groups.

3.2. Evaluation of hematological parameters

The number of white blood cells was significantly higher in the grape farmers than the other two farmer groups, whereas the onion farmers revealed higher red blood cells count and lower platelet count than the other two farmer groups (Table 2). In addition, lower basophil percentage in the grape or rose farmers and lower eosinophil proportion in the onion farmers were significantly observed when compared with those of the other farmer groups, respectively.

Table 2
Comparison of hematologic parameters among onion, grape, and rose farmers

Parameters	Onion farmers	Grape farmers	Rose farmers
WBC (10^3/μL)	3.7 ± 2.1*	6.9 ± 1.2	5.9 ± 1.7*,†
RBC (10^6/μL)	6.5 ± 0.6	4.5 ± 0.5†	4.7 ± 0.5†
Platelet (10^3/μL)	89.7 ± 35.3	231.7 ± 70.5†	233.0 ± 59.1†
Neutrophil (%)	57.6 ± 10.8	56.4 ± 9.9	50.5 ± 9.3†
Lymphocyte (%)	31.9 ± 9.9	31.1 ± 7.4	36.7 ± 9.6*
Monocyte (%)	4.8 ± 0.9	6.4 ± 1.6†	5.5 ± 1.7
Eosinophil (%)	3.4 ± 2.6	3.8 ± 2.5	4.5 ± 1.9†
Basophil (%)	0.85 ± 0.39*	0.54 ± 0.23	0.91 ± 0.50*

RBC, red blood cell; WBC, white blood cell. Data expressed as mean ± SD.
* Significant differences ($p < 0.05$) when compared with grape farmers.
† Significant differences ($p < 0.05$) when compared with onion farmers.

Table 3
Proportion (%) of lymphocyte subsets among onion, grape, and rose farmers

Immune cell (%)	Gender	Onion farmers	Grape farmers	Rose farmers
NKT cell	Female	2.7 ± 1.5	2.2 ± 0.7	1.3 ± 0.7*
	Male	0.5 ± 0.1*	2.4 ± 0.4	0.7 ± 0.3*
	Subtotal	1.6 ± 0.8*	2.3 ± 0.3	1.0 ± 0.4*
NK cell	Female	22.1 ± 4.2*	6.1 ± 2.7	28.8 ± 3.4*
	Male	30.7 ± 4.2	23.4 ± 3.6	24.8 ± 4.1
	Subtotal	26.4 ± 3.1	19.8 ± 3.3	26.9 ± 2.6
$CD8^+$ cell	Female	14.6 ± 1.4	13.7 ± 4.3	20.5 ± 2.7
	Male	16.0 ± 2.7	18.1 ± 2.6	20.2 ± 2.4
	Subtotal	15.3 ± 1.5	17.2 ± 2.2	20.4 ± 1.8†
$CD4^+$ cell	Female	34.0 ± 2.8	35.0 ± 6.9	34.0 ± 2.1
	Male	30.1 ± 2.6	31.6 ± 1.7	36.0 ± 2.2
	Subtotal	32.3 ± 1.9	32.3 ± 1.9	35.0 ± 1.5
B cell	Female	2.9 ± 0.4*	6.1 ± 1.7	2.5 ± 0.4*
	Male	1.3 ± 0.2	2.4 ± 0.4	3.1 ± 0.7†
	Subtotal	2.1 ± 0.3	3.2 ± 0.6	2.8 ± 0.4

NK, natural killer; NKT, natural killer T. Data are expressed as the mean ± SEM.
* Significant differences ($p < 0.05$) when compared with grape farmers.
† Significant differences ($p < 0.05$) when compared with onion farmers.

3.3. Proportion of lymphocyte subpopulation in peripheral blood

NKT cell proportion was significantly upregulated in the grape farmers, which is greatly imparted because of NKT values in male farmers (Table 3). A significantly fourfold lower NK cell proportion (grape farmers: 6.1 ± 2.7%; onion farmers: 22.1 ± 4.2%; and rose farmers: 28.8 ± 3.4%) and higher B cell percentage were observed in the female grape farmers. No significant differences in helper and cytotoxic T cell percentage were found within each gender, but notably, a low proportion of NK along with upregulated NKT and B cell proportion resulted from the grape farmer group.

3.4. Cytokine secretion by ex vivo–activated peripheral T cell

The PBMCs from grape farmers demonstrated upregulation of IL-13, a representative T_H2 cytokine, production compared with the other two farmer groups, mainly attributed to male farmers (Fig. 1B), whereas the level of IFN-γ showed no significant differences (data not shown) among the groups. The level of IL-4, another T_H2 cytokine, was also significantly higher to grape farmers than rose farmers (Fig. 1A), which was also mainly attributed to male farmers. A downregulated profiling of IFN-γ:IL-13 ratio was significantly demonstrated to the grape farmers when compared with the onion and the rose farmers (Fig. 1C), which could be suggestive of the T_H2 immune skewedness in grape farmers (Fig. 1A and B). The level of TNF-α, a representative proinflammatory cytokine, was also significantly elevated in grape farmers in

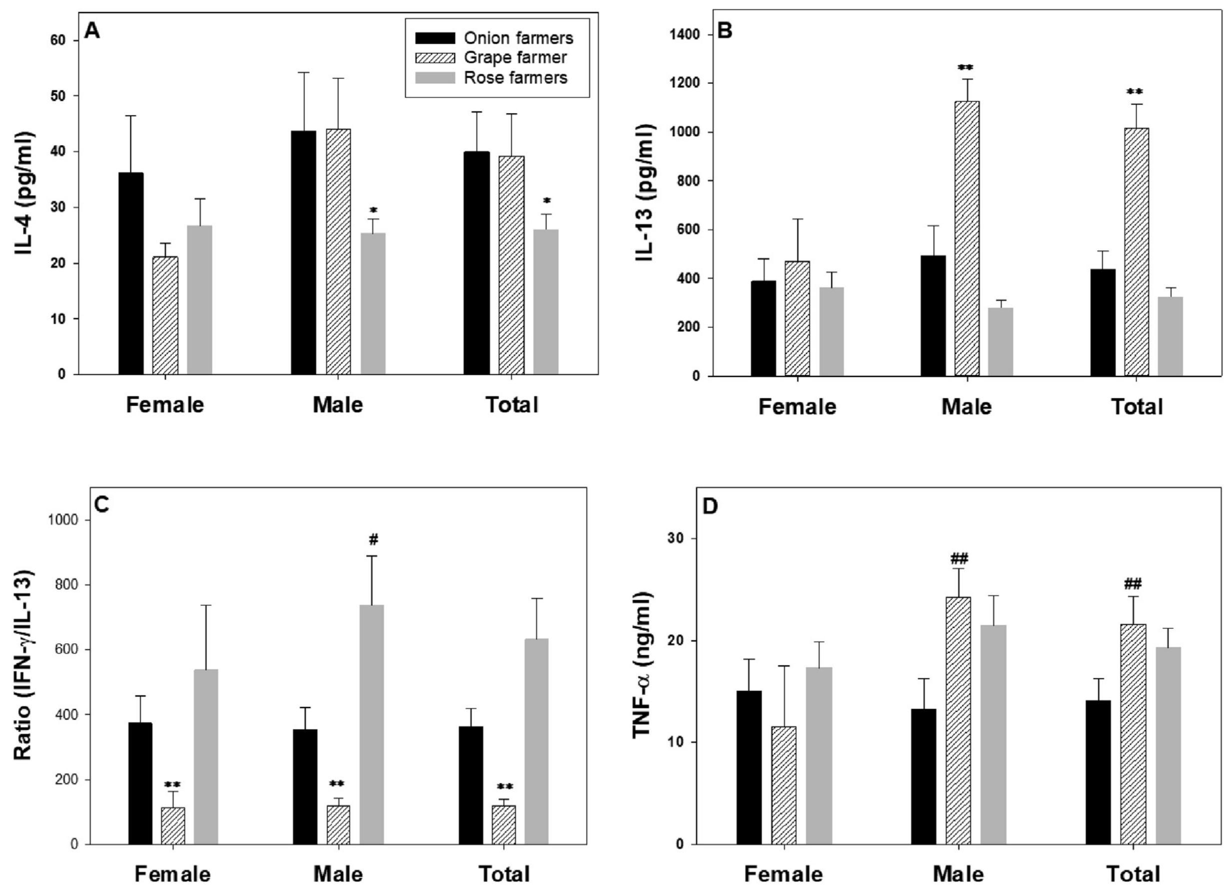

Fig. 1. Levels of cytokines produced in culture supernatants of *ex vivo* stimulated peripheral mononuclear cells from onion, grape, and rose farmers. The IFN-γ/IL-13 ratio was calculated by dividing the amount of IFN-γ by the amount of IL-13 in the same culture supernatant. Data are expressed as means ± SEMs. Statistically significant differences ($p <$ 0.05) compared with *grape farmers (A); **onion, and rose farmers (B & C); #onion farmers (C); ##onion farmers (D).

comparison to onion farmers because of remarkably high production among male farmers (Fig. 1D).

3.5. Influence on humoral immunity

Plasma IgG1 and IgG4 levels were the lowest in the grape farmers, which are mainly attributed to male farmers (Fig. 2A and B). Meanwhile, IgE level in the grape farmers, which elevation is a hallmark of allergic reactions, such as asthma, allergic rhinitis, and anaphylaxis [15,16,21], was not significantly different from that of the onion or the rose farmers (Fig. 2C). A significantly lower IgA level was demonstrated in the onion farmers than the grape or the rose farmers (Fig. 2D).

4. Discussion

Because of the global population growth, changing dietary preferences, or biofuel demand, agricultural intensification has been used to increase agricultural production worldwide [27,28]. Correspondingly, a rise in the prevalence of ill health outcomes such as respiratory illness, allergy, pesticide poisoning, musculoskeletal disorders, neurologic diseases, mental illness, or cancer has been reported among agricultural workers in Korea [29,30]. Although exposure agent characterization is quite challenging in crop farming, few studies have reported certain exposure measurements during specific agricultural tasks, such as harvesting or soil preparation in the orchards and open-field farms [7,9]. PM concentrations and personal dust exposure levels during soil management activities as land planning, discing, and sowing in open-field farming were reported to exceed the threshold limits, and the presence of cabin and ventilation system in tractors was found vital in determining personal dust exposure among the operators [29]. On the other hand, the report on grape and citrus orchards showed significantly high organic and inorganic dust exposure among farmworkers, and high foliar dust exposure appeared as a significant determining factor in exposure during manual harvesting [19]. Also, the maximum application of pesticides with speed sprayers in fruit farming than rice and vegetable farming has been reported in Korea [8]. Similarly, in greenhouses, indoor microclimate conditions such as ventilation, temperature, humidity, pesticide exposure, and activities such as spraying or mist cooling have been associated with health effects among the workers [30]. Hence, these potentially hazardous exposures in crop farms could potentially cause negative health impacts on the workers.

Agricultural workers are at risk for respiratory illnesses and various allergic diseases following recurrent exposure to various agents as dust, pollens, mites, or pesticides [10,31]. Major pulmonary symptoms such as coughing, phlegm, and chest tightness have been observed in tractor operators, and the relation to the smoking habit, use of personal protective equipment (PPE), and availability of cabin in tractors were also noted [18]. Diseases such as asthma, pulmonary fibrosis, and lung cancer are associated with dust inhalation, but organic dust exposure is often related to allergic responses as asthma, whereas inorganic dust exposure promoted diseases as bronchitis and pneumoconiosis [10,31,32]. The prevalence of allergic asthma, rhinitis, or work-related respiratory symptoms as wheezing, rhinorrhea, and nasal itching were also

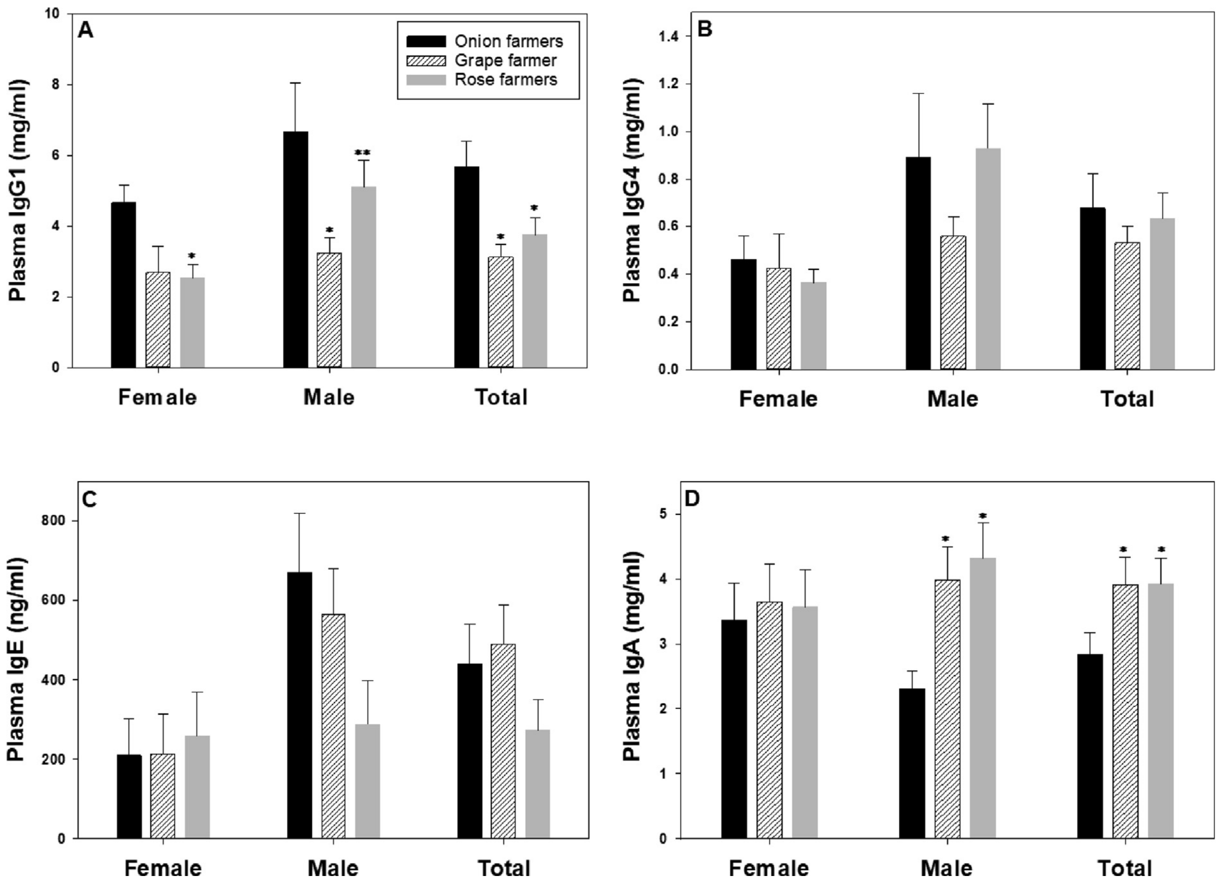

Fig. 2. Levels of plasma immunoglobulins in onion, grape, and rose farmers. Data represented as means ± SEMs. Statistically significant differences ($p < 0.05$) compared with *onion farmers (A,D); **grape farmers (A).

reported primarily in orchard and greenhouse farmworkers [20,33,34].

Allergic diseases have been long linked with enhanced T_H2 polarization along with decreased T_H1 response [35], and organic dust-mediated allergic-immune alteration characterized by T_H2 predominance has been demonstrated in livestock husbandry workers [15,16]. In similarity, our finding on upregulation of T_H2 cytokine production among grape farmers especially male subpopulation could be illustrative of propensity to allergic response in orchard farmers. IL-4 and IL-13 function as the two main cytokines mediating T_H2 response, but they exhibit different expression patterns, which determine their distinctive functions in allergic immunity. IL-4 plays a major role in humoral response, whereas IL-13 mediates tissue responses such as eosinophil recruitment [36]. Furthermore, it is proved that IL-13 is produced by type 2 innate lymphoid cell (ILC2) and influences $CD4^+$ T cell differentiation and activation to T_H2 phenotype [37]. This phenomenon might suggest the predominantly high IL-13 levels than IL-4 among grape farmers when compared with the onion and rose farmers. Also, the repertoire of ILC2 and T_H2 response for excessive IL-13 production has been observed with type 2 inflammation in asthma and allergic diseases [38,39]. These observations may lead to the prognosis of T_{H2}-mediated allergic reactivity followed by inflammation in grape farmers. In addition, NKT cells in humans account for the upregulated production of T_H2 cytokines and promotion of allergy despite their paradoxical biological function in cell-mediated immunity [40]. In accordance, our findings also parallelly revealed the elevated T_H2 cytokine and NKT cell proportion among grape farmers.

Following the recent finding on the higher prognostic value of IFN-γ:IL-13 ratio in determining skewed immune reactivity, we analyzed IFN-γ:IL-13 ratio in our study to assess T_H1/T_H2 immune balance [41] and found that immune reactivity was skewed to T_H2 response among grape farmers followed by onion and the rose farmers. Hereby, our finding on allergic predominance potential among grape farmers correspondingly correlated with the previous observation eliciting skewed T_H2 responses in allergic diseases as airway allergies, skin sensitizations, and gastrointestinal allergies [25,42,43]. On the contrary, our finding on T_H2 skewed immune response showed the least changes in humoral markers among grape farmers. This could be supported by the finding of a study on airway inflammation revealing systemic augmentation of allergy-induced inflammation in airways by IL-13 independent of IgE and IgA [44]. This overall observation on the predominance of T_H2 immune response among grape farmers could be primarily related to the excess foliar dust exposure, pesticide usage, or other agricultural work patterns in the orchards [8,9,19].

This study noted that onion farmers tended to have a higher level of plasma IgG1, IgG4, and IgE followed by grape and rose farmers. Notably, these immunoglobulins are associated with a high risk for allergic conditions [45]. These elevated humoral markers significantly noted in onion farmers could be suggestive of possible recurrent allergen exposure accompanied by the presence of allergen-specific antibodies and/or peripheral T cell tolerance [23]. In addition, IgE memory cells were found to be produced by somatically hypermutated or high-affinity antigen-experienced IgG^+ precursor cells, which supports the finding that humoral markers in the onion farmers were elevated despite the low T_H2

cytokine expression compared with grape farmers. Contrastingly, plasma IgA, responsible for mucosal immunity, was significantly low in male onion farmers. Our finding on circulating IgA level and low eosinophil proportion in onion farmers coincided with the fact that eosinophils promote the generation and maintenance of IgA expressing plasma cells [46] while contrasted with the elevated IgE level among onion farmers. Moreover, the shorter half-life of IgA could be an important contributing factor for declined IgA in onion farmers [47].

The majority of our findings was found prevalent in the male subpopulation of grape and onion farmers. This gender disparity could probably involve the usage of farm machinery, the extent of agricultural activity participation, and differences in farm activities carried out [48]. Nevertheless, confounding factors as personal habits such as smoking and dietary habits, use of PPE, and activity pattern could play an eminent role in determining the immune status of individuals. As the average age and working duration of our study populations had no significant differences, we consider that age and working duration do not stand as confounding factors in our result. Meanwhile, smoking duration was significantly different between onion farmers and rose farmers without significant differences with grape farmers. Therefore, smoking duration as a confounding factor may not substantially influence on the prominent cell-mediated allergic-immune profile in the grape farmers.

In summary, we noted a significant difference in overall immune status among workers at various crop farms. Grape orchard farmers revealed the prominent cell-mediated allergic-immune profile, whereas rose greenhouse farmers demonstrated the minimum immune alteration when compared with grape and onion farmers. As each one crop type was allocated to three different farming systems in this study, it is suggested that more studies on various crops belonging to different farming types could be conducted to reveal more significant observations. To our knowledge, the present investigation stands as the first study to demonstrate differences in workplace-related overall immune status in three different agricultural crop farm groups, namely, grape orchard, rose greenhouse, and open-field onion farm. However, concerning certain limitation in the study including not sufficient number of subjects investigated or no data on exposure assessment of various hazardous agents, the present study results should be carefully interpreted and better considered to be a pilot study. Although farmers are generally exposed to a composite mixture of agents, few agents are measured in most studies, and it is unclear whether these associated could be confounded by exposure to other relevant agents as well. Therefore, to further validate these findings and elucidate the mechanism by which differing farm exposure shows the altered immune responses in workers, systematic studies involving large study populations from different farming systems with subsequent exposure assessment need to be done.

Conflicts of interest

All authors have no conflicts of interest to declare.

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