# Oxidative stress and subclinical atherosclerosis in agricultural workers:

## a nested case control study

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# Oxidative stress and subclinical atherosclerosis in agricultural workers:

## a nested case control study

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# ABSTRACT

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**Background and objectives**

Numerous studies examining the complex pathophysiological mechanisms underlying cardiovascular events have revealed that oxidative stress plays a critical role in atherogenesis. The aim of this study was to investigate the association between oxidative stress and carotid artery intima-media thickness (CIMT) as an indicator of subclinical atherosclerosis in agricultural workers.

**Subjects and methods**

We identified 51 case participants (CIMT level ≥ 0.9 mm) who were enrolled during a visit to any cohort center between May 1, 2011, and August 31, 2011, and who had never had a cardiovascular event such as angina, myocardial infarction, or stroke. We randomly recruited 51 control participants (CIMT level < 0.9 mm) during the same period using frequency matching by age group. For oxidative stress markers, we measured the levels of 8-hydroxy 2′-deoxyquuanosine (8-OHdG), malondialdehyde (MDA), and 8-iso-prostaglandin F2α (isoprostane). To assess pesticide exposure, we calculated the lifetime exposure to pesticides in

months. Conditional logistic regression models were used to evaluate the relative relationships between occupational history and oxidative stress markers in the presence of subclinical atherosclerosis.

**Results**

Compared with general workers and low oxidative stress strata, agricultural workers and high oxidative stress strata showed an overwhelmingly increased risk and odds ratios (ORs) of subclinical atherosclerosis beyond the sum of the ORs for the other strata (OR [95% confidence interval], 16.00 [3.99–64.18] for isoprostane, 10.40 [3.07–35.28] for MDA, and 8.64 [2.57–29.07] for 8-OHdG). In path analysis, pesticide exposure had a direct effect on the increase of oxidative stress levels ($\beta = 0.425$ for isoprostane, $\beta = 0.396$ for MDA, and $\beta = 0.264$ for 8-OHdG) and an indirect effect on CIMT ($\beta = 0.146$ for model A and $\beta = 0.161$ for model B). Oxidative stress had a direct effect on CIMT ($\beta = 0.248$ for isoprostane, $\beta = 0.227$ for MDA, and $\beta = 0.328$ or 0.330 for 8-OHdG).

**Conclusion**

We observed an increased risk of developing subclinical atherosclerosis in agricultural workers who had higher levels of oxidative stress than general workers. In addition, the higher levels of oxidative stress were correlated with pesticide exposure. The deducible pathologic links between agricultural workers and their higher risk of subclinical atherosclerosis could be explained by the indirect effect of pesticide exposure on oxidative stress levels.

Key words: Oxidative stress, Agricultural workers, Subclinical atherosclerosis, Carotid intima media thickness.

# I. Introduction

Atherosclerosis and endothelial dysfunction are considered as underlying mechanisms of cardiovascular disease. Numerous studies examining the complex pathophysiological mechanisms associated with cardiovascular disease have revealed a critical role for oxidative stress in the development of atherosclerosis [1].

Oxidative stress is caused by the overproduction of reactive oxygen species (ROS) and free radicals beyond the physiological detoxifying ability of cells or their ability to repair the resulting damage [2]. Although ROS and free radicals are essential elements of biological systems, such as cell signaling, controlling vascular tone, and the generation and degeneration of target cells [3], their high biological reactive properties cause the oxidative damage of lipids, DNA, and proteins. However, the direct measurement of free radicals and ROS using electron resonance or spin trapping is very problematic and expensive in humans [4]. Therefore, simpler methods, which examine the end products of oxidative damage, are used to evaluate oxidative stress. For example, 8-hydroxy 2′-deoxyguanosine (8-OHdG) is a marker of damaged proteins and DNA [5], while malondialdehyde (MDA) and 8-iso-prostaglandin F2α (isoprostane) are markers of lipid peroxidation damage [3,6]. These forms of oxidative damage are

considered as key pathologic mechanisms underlying chronic diseases such as cardiovascular disease [7].

In Korea, agricultural workers have a higher prevalence of cardiovascular disease than the general population [8]. In agricultural workplaces, several environmental toxic materials are used, such as organic solvents and pesticides, and they are pathologically linked with oxidative stress [9,10]. In addition, the levels of oxidative stress are higher in workers who apply pesticides than in controls [11,12]. ROS and the alteration of the antioxidant status have been implicated in the toxicity of pesticides [13], and increasing lifetime days of pesticide exposure are associated with chronic diseases caused by high oxidative stress levels [14]. However, these studies did not perform further analyses such as examining the connection between lifetime days of pesticide exposure, elevated levels of oxidative stress, and clinical disease, e.g., atherosclerosis. To investigate such a connection, including the use of path analysis, we measured carotid intima media thickness (CIMT) as a surrogate marker of subclinical atherosclerosis [15] and estimated the lifetime days of pesticide exposure.

Hence, we aimed to elucidate the role of oxidative stress and pesticide exposure in the development of atherosclerosis in agricultural workers. The findings of this study could have important clinical significance for preventing the development of atherosclerosis in agricultural workers.

## II. Methods

### A. Study subjects

The current study was nested within the Korean Genomic Rural Cohort Study (KGRC), which includes almost 57% agricultural workers in the total male cohort. All participants provided signed informed consent for their participation according to the requirements of the Institutional Review Board of Wonju Christian Hospital.

We enrolled male participants during any visit to a cohort center between May 1, 2011 and August 31, 2011. During this period, a total of 478 individuals (male, 214; female, 264) visited the cohort centers. A medical history questionnaire was used to screen for the exclusion criteria. We excluded 40 participants who had a cardiovascular event, e.g., angina, myocardial infarction, and stroke, or a medical history of chronic hepatitis, osteoporosis, kidney disease, asthma, or any malignant disease. Using a CIMT cut-off level of 0.9 mm [16], 51 case participants (CIMT level $\geq$ 0.9 mm) and 51 control participants (CIMT level $<$ 0.9 mm) were assigned at random, with frequency matching by age group (41–50, 51–60, 61–70, and 71–80 years).

### B. Occupational history and pesticide exposure assessment

To differentiate agricultural workers from general workers, we used the "Korean Standard Job Classification (KSJC)" questionnaire. The KSJC consists of 52 sub-categories for a total of 10 occupation groups. The 6$^{th}$ categories consists of agricultural, fishery, and forestry workers. The 1$^{st}$ sub-category of the 6$^{th}$ category is agricultural workers. When the participants were unemployed or had been involved in 2 or more occupations in their lifetime, the job that was performed the longest was chosen as their occupational history. To assess pesticide exposure in agricultural workers, we estimated their total lifetime exposure to pesticides in months. The lifetime exposure to pesticides (pesticide exposure month, PEM) was determined by multiplying the years of pesticide use by the average days of use per year and then dividing by 30 [14]. To compare the risk of developing subclinical atherosclerosis in the low or high pesticide exposure groups with general workers, we defined the low and high pesticide exposure groups according to their median level.

### C. Measurement of anthropometrics, metabolic characteristics, and oxidative stress markers

According to standard procedures [17], comprehensive questionnaires and physical examinations were performed. A history of regular alcohol consumption was taken. Current smoker, ex-smoker, and never smoked were categorized and

the percent of never smoked was chosen as the variable for smoking history. Data regarding the following parameters were obtained from a self-reported questionnaire: medical history of cardiovascular events, e.g., angina, myocardial infarction, and stroke; medical history of chronic hepatitis, osteoporosis, kidney disease, asthma, or any malignant disease; and pharmacological treatment of hypertension (HTN), diabetes mellitus (DM), and dyslipidemia.

Body weight, height, and waist circumference were measured while wearing indoor clothing without shoes. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice from the right arm using a standard mercury sphygmomanometer (Baumanometer; USA). The mean SBP and DBP levels were used for data analysis. Venous blood samples were drawn from the participants in the morning after they fasted overnight, and the samples were stored immediately at -80℃. Fasting blood glucose (FBG) and insulin (FBI) were determined by a glucose oxidase-based assay and double-antibody radioimmunoassay (RIA). The serum concentrations of low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides were determined by enzymatic methods (ADVIA 1650; Bayer, USA).

The concentrations of 8-OHdG, isoprostane, and MDA were measured in spot urine samples using a high-performance-liquid chromatography-triple tandem mass detector (HPLC-MS/MS) machine (Agilent 6410; Agilent). For 8-OHdG, the method detection limits (MDL) was 0.053 μg/L, and the relative standard deviation (RSD) was 1.29 %, 0.90 % for accuracy and repeatability. For

isoprostane, MDL was 0.162 pg/ml, and RSD was 1.29% for accuracy, and 2.10% for repeatability. For MDA, MDA was 0.044 μmol/L, RAD was 5.36% for accuracy, and 2.10% for repeatability.

### D. Ultrasound imaging analysis

We used a B-mode ultrasound system (Vivid 7; General Electric-Vingmed) with a 12-MHz transducer to determine the thickness of the bilateral carotid intima media in both sides. We measured CIMT on a longitudinal 2-dimentional ultrasonography image. Still images from near the carotid bifurcation were digitally acquired and the far walls of the carotid artery were displayed as 2 bright lines separated by a hypoechoic space. CIMT between the leading edge of the first bright line (lumen-intima interface) and the leading edge of the second bright line (media-adventitia interface) was detected using semi-automated edge-detection software. More than 2 cm lengths of CIMT were taken from 1 cm of the carotid bulb. The mean maximum level of both sides CIMT was chosen as an indicator of subclinical atherosclerosis [15].

### E. Statistical Analysis

The data were expressed as frequencies (%), mean values with SD, and median values with the low and high quartiles. The distribution of continuous

variables was examined for skewness and kurtosis and was logarithmically transformed, when appropriate. We used t-test, Mann-Whitney U test, or chi-square test to compare the difference between case and control groups. Age-adjusted Pearson's correlation analysis was used between the CIMT and oxidative stress markers. Multiple conditional logistic regression models were tested for the ORs of oxidative stress markers and occupational history with an adjustment for the pharmacological treatment of HTN, DM, and dyslipidemia, as well as other metabolic risk factors, e.g., body mass index (BMI), smoking history, alcohol consumption, and the presence of metabolic syndrome (MetS). In multiple conditional logistic regression models, the tertile (T) increment of isoprostane, 8-OHdG, and MDA was used as an independent variable (isoprostane [ng/mg creatinine]: $1^{st}$ T, <0.21; $2^{nd}$ T, 0.21–0.51; $3^{rd}$ T, ≥0.51; 8-OHdG [μg/g creatinine]: $1^{st}$ T, <0.66; $2^{nd}$ T, 0.66–1.25; $3^{rd}$ T, ≥1.26; MDA [μmol/g creatinine]: $1^{st}$ T, <0.10; $2^{nd}$ T, 0.10–0.20; $3^{rd}$ T, ≥0.20). Analysis of covariance (ANCOVA) designs, adjusted for anthropometric characteristics and pharmacological treatment history for HTN, DM, and dyslipidemia, were used to compare the levels of oxidative stress markers between agricultural and general workers. To elucidate the relative relationships between occupational history and oxidative stress markers in subclinical atherosclerosis, oxidative stress markers were transformed into dichotomous variables using their median levels. Therefore, 2 × 2 strata were created, and the reference strata consisted of low oxidative stress marker levels and the general worker group. The other strata consisted of low oxidative stress

marker levels and the agricultural worker group, and high oxidative stress marker levels and the general worker group or agricultural worker group. These associations are expressed on the figures with an OR and the '*' symbol, which indicates a *P*-value < 0.05. We undertook path analysis to assess the connection between pesticide exposure and oxidative stress on the development of subclinical atherosclerosis. First, we estimated the correlation matrix, mean, and SD among CIMT, isoprostane, 8-OHdG, MDA, and PEM. Second, linear regression analysis with backward selection was used to select the variables that were included in the models. Third, regression analysis was performed using path models. The input data were in the form of a correlation matrix and SD. Finally, we modified the path analysis model according to the model fit indices, such as chi-square value with degrees of freedom, comparative fit index (CFI), and root mean square error of approximation (RMSEA). The standardized coefficients (β) are listed on the model paths. Statistical significance was determined at $P < 0.05$ for all comparisons in the current study.

# III. Results

## A. Anthropometric and metabolic characteristics of the case and control groups

We used frequency matching according to the age group distribution in the case group. The distributions of the age groups are presented in Table 1. The smoking history and pharmacological treatment of HTN, DM, and dyslipidemia were similar for the 51 cases and 51 controls. Various metabolic biomarkers, such as triglyceride, FBG, FBI, SBP, and DBP were similar between case and control groups. The serum level of LDL was higher in the case group than in the control group. The levels of all oxidative stress markers were significantly higher in the case group than in the control group (median value of case vs. control, respectively: isoprostane, 0.50 vs. 0.21, $P < 0.0001$; 8-OHdG, 1.26 vs. 0.74, $P = 0.0021$; MDA, 0.20 vs. 0.11, $P = 0.0005$). There were significantly more agricultural workers in the case group (74.51%) than in the control group (49.02%).

Table 1. Demographic and metabolic characteristics of the case and control groups

| | Cases (n=51) CIMT ≥0.9 mm | | Controls (n=51) CIMT < 0.9 mm | | *P* value |
|---|---|---|---|---|---|
| ***Demographic characteristics*** | | | | | |
| Age, n (%) | | | | | 1.000 |
| 40~50 | 2 | (3.92) | 2 | (3.92) | |
| 51~60 | 14 | (27.45) | 14 | (27.45) | |
| 61~70 | 29 | (56.86) | 29 | (56.86) | |
| 71~80 | 6 | (11.76) | 6 | (11.76) | |
| Type of Occupations, n (%) | | | | | |
| Agriculture workers | 38 | (74.51) | 25 | (49.02) | |
| General workers | 13 | (25.49) | 26 | (50.98) | 0.0081 |
| Pharmacologic treatments, n (%) | | | | | |
| Hypertension | 18 | (35.29) | 20 | (39.22) | 0.6821 |
| Diabetes | 6 | (11.76) | 5 | (9.08) | 0.7496 |
| Dyslipidemia | 6 | (11.76) | 9 | (17.65) | 0.4016 |
| % of never smoker, n (%) | 19 | (37.25) | 24 | (47.06) | 0.4239 |
| % of alcohol drinker, n (%) | 34 | (66.67) | 35 | (68.63) | 0.8324 |
| ***Metabolic characteristics*** | | | | | |
| Isoprostane / creatinine ratio | 0.50 | (0.27-0.80) | 0.21 | (0.15-0.47) | <.0001 |
| 8-OHdG / creatinine ratio | 1.26 | (0.69-1.83) | 0.74 | (0.45-1.14) | 0.0021 |
| MDA / creatinine ratio | 0.20 | (0.13-0.28) | 0.11 | (0.08-0.16) | 0.0005 |
| Triglyceride (mg/dL) | 133.00 | (97-177) | 128.00 | (84-159) | 0.2955 |
| Body mass index (kg/m2) | 23.98 | ±2.73 | 24.20 | ±2.93 | 0.6935 |
| Waist circumference (cm) | 85.25 | ±5.78 | 86.11 | ±7.71 | 0.5289 |
| Fasting plasma glucose (mg/dL) | 104.92 | ±24.73 | 98.75 | ±14.23 | 0.1290 |
| Fasting plasma insulin (μIU/mL) | 6.66 | ±2.52 | 7.32 | ±3.65 | 0.2879 |
| Systolic blood pressure (mmHg) | 127.48 | ±14.55 | 126.88 | ±14.92 | 0.8390 |
| Diastolic blood pressure (mmHg) | 76.94 | ±11.72 | 80.59 | ±9.55 | 0.0893 |
| LDL (mg/dL) | 117.58 | ±25.94 | 106.02 | ±31.07 | 0.0453 |
| HDL (mg/dL) | 50.02 | ±9.89 | 51.65 | ±12.44 | 0.4692 |
| MetS proportion (n, %) | 17 | (33.30) | 18 | (35.29) | 0.8348 |

Data are expressed as the median value (inter-quartile range) using the Mann-Whitney U test, mean ± standard deviation with the t-test, or number (%) with the chi-square test

Abbreviations: Isoprostane, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; MDA, malondialdehyde; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; MetS, metabolic syndrome.

## B. Age-adjusted Pearson's correlation coefficient between CIMT and oxidative stress markers

The relationships between CIMT levels and the oxidative stress markers are shown in Table 2. The CIMT levels were significantly related to the levels of isoprostane (r = 0.313, $P = 0.0017$), MDA (r = 0.243 $P = 0.0480$), and 8-OHdG (r = 0.195, $P = 0.0492$). BMI was not correlated with the levels of isoprostane, 8-OHdG, and MDA. The levels of MDA were correlated with the isoprostane (r = 0.400, $P < 0.0001$) and 8-OHdG (r = 0.288, $P = 0.004$) levels, but there was no significant correlation between the levels of isoprostane and 8-OHdG.

Table 2. Age-adjusted Pearson's correlation coefficient between subclinical atherosclerosis and oxidative stress biomarkers

| | CIMT | BMI | Isoprostane | 8-OHdG | MDA |
|---|---|---|---|---|---|
| CIMT | 1 | | | | |
| BMI | 0.026 | 1 | | | |
| Isoprostane | 0.313* | -0.089 | 1 | | |
| 8-OHdG | 0.195* | 0.034 | 0.180 | 1 | |
| MDA | 0.243* | -0.134 | 0.400* | 0.288* | 1 |

*$P < 0.05$

Abbreviations: CIMT, carotid intima media thickness; BMI, body mass index; isoprostane, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; MDA, malondialdehyde.

### C. Occupational history of agricultural workers and the risk of subclinical atherosclerosis with oxidative stress

The OR for subclinical atherosclerosis in agricultural workers (OR, 95% confidence interval [CI]: 3.04, 1.23–7.91) was higher than in general workers (Table 3). This significant relationship was maintained after adjustment for age, LDL, pharmacological treatment for HTN, DM, and dyslipidemia, BMI, smoking history, alcohol consumption, and MetS (OR, 95% CI: 3.43, 1.29–9.09). A further adjustment for oxidative stress attenuated these relationships (in models B and C), with the relationship between occupational history and subclinical atherosclerosis losing its significance. Compared with the 1$^{st}$ tertile for isoprostane and MDA, the 2$^{nd}$ and 3$^{rd}$ tertiles of these oxidative stress markers were associated with an increased risk of subclinical atherosclerosis in the univariate model (ORs [95% CI] for isoprostane: 2$^{nd}$ tertile, 3.80 [1.36–10.59]; 3$^{rd}$ tertile, 7.30 [2.50-21.29]; ORs [95% CI] for MDA: 2$^{nd}$ tertile, 3.08 [1.13-8.42], 3$^{rd}$ tertile, 6.39 [2.24–18.25]). The 3$^{rd}$ tertile of 8-OHdG was a high risk factor compared with the 1$^{st}$ tertile (ORs [95% CI] for 8-OHdG: 2$^{nd}$ tertile, 1.34 [0.48-3.78], 3$^{rd}$ tertile, 5.43 [1.86–15.84]). The oxidative stress markers for lipid damage, i.e., isoprostane and MDA, and DNA damage, i.e., 8-OHdG, were independently associated with subclinical atherosclerosis when they were incorporated into models B (I) and C (I). We estimated multivariable ORs adjusted for the pharmacological treatment of HTN,

DM, and dyslipidemia, as well as LDL, BMI, smoking history, alcohol consumption, MetS, and an occupational history of agricultural work in models B (II) and C (II). In those models, these variables strengthened the associations, and the oxidative stress markers for lipid and DNA damage were still significantly associated (ORs [95% CI] for isoprostane: 2$^{nd}$ tertile, 4.15 [1.22–14.11]; 3$^{rd}$ tertile, 13.08 [3.13–54.70]; ORs [95% CI] for 8-OHdG: 2$^{nd}$ tertile, 0.85 [0.22–3.18]; 3$^{rd}$ tertile, 5.83 [1.50–22.67] in model B [II]; ORs [95% CI] for MDA: 2$^{nd}$ tertile, 1.75 [0.53–5.78]; 3$^{rd}$ tertile, 4.51 [1.25–16.30]; ORs [95% CI] for 8-OHdG: 2$^{nd}$ tertile, 1.15 [0.33–4.04], 3$^{rd}$ tertile, 4.70 [1.24–17.90] in model C [II]).

Table 3. Association between the risk of subclinical atherosclerosis and occupational history with oxidative stress

| | Univariate Model OR (95% CI) | Model A OR (95% CI) | Model B (I) OR (95% CI) | Model B (II) OR (95% CI) | Model C (I) OR (95% CI) | Model C (II) OR (95% CI) |
|---|---|---|---|---|---|---|
| ***Occupational history of*** | | | | | | |
| General workers | 1.00 - | 1.00 - | 1.00 - | 1.00 - | 1.00 - | 1.00 - |
| Agriculture workers | 3.04 (1.23-7.91) | 3.43 (1.29-9.09) | 2.42 (0.92-6.34) | 2.99 (0.88-10.20) | 2.19 (0.86-5.57) | 2.61 (0.84-8.06) |
| ***Oxidative stress of lipid damage*** | | | | | | |
| **Isoprostane, ng/mg creatinine** | | | | | | |
| 1st tertile (<0.21) | 1.00 - | | 1.00 - | 1.00 - | | |
| 2nd tertile (<0.51) | 3.80 (1.36-10.59) | | 3.97 (1.31-12.04) | 4.15 (1.22-14.11) | | |
| 3rd tertile (≥0.51) | 7.30 (2.50-21.29) | | 7.28 (2.26-23.46) | 13.08 (3.13-54.70) | | |
| **MDA, µmol/g creatinine** | | | | | | |
| 1st tertile (<0.10) | 1.00 - | | | | 1.00 - | 1.00 - |
| 2nd tertile (<0.20) | 3.08 (1.13-8.42) | | | | 2.22 (0.76-6.48) | 1.75 (0.53-5.78) |
| 3rd tertile (≥0.20) | 6.39 (2.24-18.25) | | | | 3.93 (1.27-12.14) | 4.51 (1.25-16.30) |
| ***Oxidative stress of DNA damage*** | | | | | | |
| **8-OHdG, µg/g creatinine** | | | | | | |
| 1st tertile (<0.66) | 1.00 - | | 1.00 - | 1.00 - | 1.00 - | 1.00 - |
| 2nd tertile (<1.26) | 1.34 (0.48-3.78) | | 1.21 (0.39-3.71) | 0.85 (0.22-3.18) | 1.41 (0.47-4.24) | 1.15 (0.33-4.04) |
| 3rd tertile (≥1.26) | 5.43 (1.86-15.84) | | 5.39 (1.61-18.07) | 5.83 (1.50-22.67) | 3.95 (1.24-12.60) | 4.70 (1.24-17.90) |

*OR (95% CI), odds ratio (95% confidence interval). Model A: adjusted for age, low-density lipoprotein, pharmacological treatment of hypertension, diabetes, and dyslipidemia, body mass index, smoking history, alcohol consumption, and metabolic syndrome. Models B (I) and C (I): adjusted for age. Models B (II) and C (II): Models B (I) and C (I) plus further adjustments for low-density lipoprotein, pharmacological treatment of hypertension, diabetes, dyslipidemia, body mass index, smoking history, alcohol consumption, and metabolic syndrome.

Abbreviations: isoprostane, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; MDA, malondialdehyde; OR (95% CI), odds ratio (95% confidence interval).

### D. Relative relationships between occupational history and oxidative stress markers in subclinical atherosclerosis

All of the oxidative stress marker levels were dichotomized by their median level, and we calculated the risk of subclinical atherosclerosis by considering the occupational history (Table 4). In the general workers, the ORs (95% CI) of the high oxidative stress group were higher for MDA (OR [95% CI], 5.33 [1.26–22.57]) and 8-OHdG (OR [95% CI], 4.34 [1.06–17.86]) than the ORs of the low oxidative stress group. In the agricultural workers, the ORs (95% CI) of the high oxidative stress group were higher for isoprostane (OR [95% CI], 7.69 [2.35–25.21]) and MDA (OR [95% CI], 3.25 [1.13–9.31]).

The ORs of the 2 × 2 strata created by low or high oxidative stress marker levels and the agricultural or general worker groups are shown in Table 4. Compared with the reference strata, the agricultural workers and high oxidative stress strata were at risk of subclinical atherosclerosis (ORs [95% CI]: isoprostane, 16.00 [3.99–64.18]; MDA, 10.40 [3.07–35.28]; 8-OHdG, 8.64 [2.57-29.07]). Logistic regression models were assessed after adjustment for BMI, pharmacological treatment of HTN, DM, and dyslipidemia, and the presence of MetS. Compared with the reference strata, the agricultural workers and high oxidative stress strata showed an overwhelming increased risk and ORs of subclinical atherosclerosis beyond the sum of the ORs of the other strata for isoprostane (Figure 1) (ORs [95% CI]: isoprostane, 16.86 [4.09–69.60]; MDA,

12.13 [3.24–15.45]; 8-OHdG, 14.53 [3.44–61.45]). There was no significant interaction between occupational history and oxidative stress markers (*P* for interaction: *P* = 0.2606 for isoprostane; *P* = 0.4623 for MDA; *P* = 14.53 for 8-OHdG, not shown in the figure).

Table 4. Relative relationships between occupational history and oxidative stress markers in subclinical atherosclerosis

| Occupational history of agriculture work | Oxidative stress | | Cases (No.) | Controls (No.) | *OR | (95%CI) | †OR | (95%CI) |
|---|---|---|---|---|---|---|---|---|
| Absent | Isoprostane (at 0.302 ***ng/mg creatinine )*** | Low | 5 | 16 | 1.00 | reference- | 1.00 | - |
| | | High | 8 | 10 | 2.56 | (0.65-10.06) | 2.56 | (0.65-10.06) |
| Present | | Low | 13 | 20 | 1.00 | - | 2.08 | (0.61-7.07) |
| | | High | 25 | 5 | 7.69 | (2.35-25.21) | 16.00 | (3.99-64.18) |
| Absent | MDA (at 0.136 ***µmol/g creatinine)*** | Low | 5 | 20 | 1.00 | - | 1.00 | - |
| | | High | 8 | 6 | 5.33 | (1.26-22.57) | 5.33 | (1.26-22.57) |
| Present | | Low | 12 | 15 | 1.00 | - | 3.20 | (0.93-11.05) |
| | | High | 26 | 10 | 3.25 | (1.13-9.31) | 10.40 | (3.07-35.28) |
| Absent | 8-OHdG (at 0.897 ***µg/g creatinine)*** | Low | 5 | 19 | 1.00 | - | 1.00 | - |
| | | High | 8 | 7 | 4.34 | (1.06-17.86) | 4.34 | (1.06-17.86) |
| Present | | Low | 13 | 14 | 1.00 | - | 3.53 | (1.02-12.21) |
| | | High | 25 | 11 | 2.45 | (0.87-6.90) | 8.64 | (2.57-29.07) |

Abbreviations: isoprostane, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; MDA, malondialdehyde; OR, odds ratio; 95% CI, 95% confidence interval.
*reference group is the low oxidative stress strata for each occupational history.
†reference group is the low oxidative stress strata and absence of an occupational history of agricultural work.

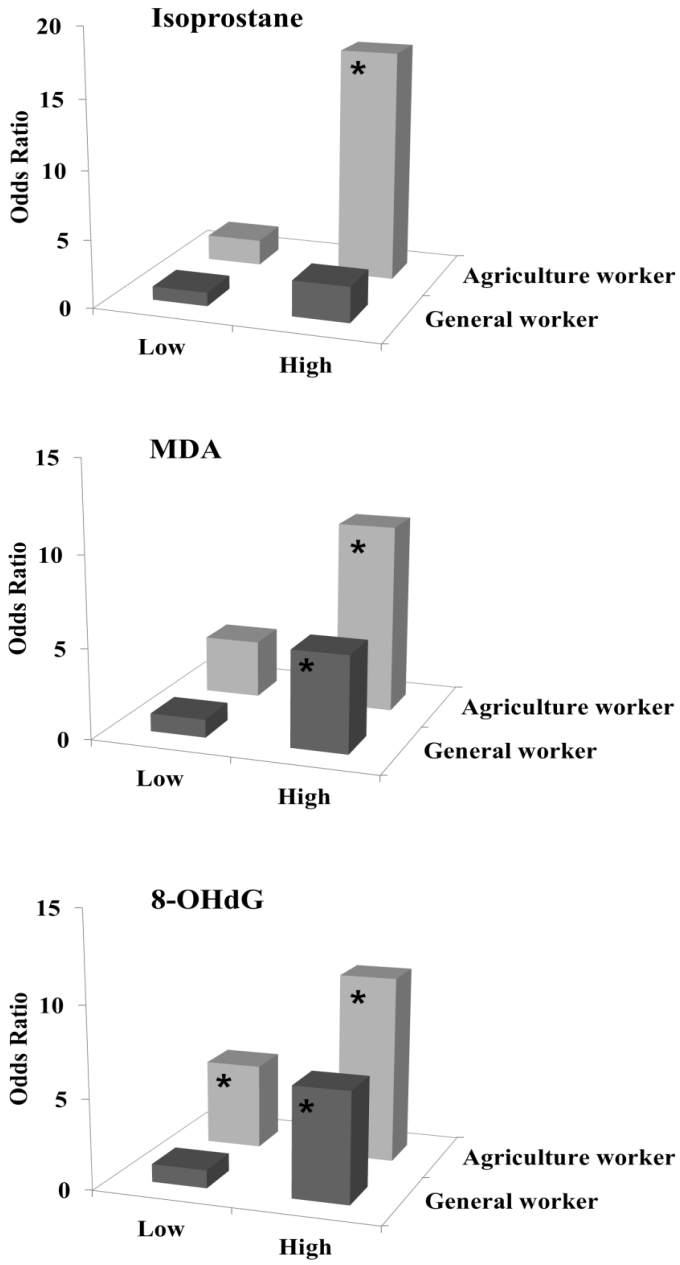


**Fig. 1. Relative relationships between occupational history and oxidative stress markers in subclinical atherosclerosis.**

***indicates a statistically high odds ratio compared with the reference strata, which include low oxidative stress markers and general workers. "Low" or "High" indicates the level of oxidative stress markers below or above the median level, respectively. All models were adjusted for age, low-density lipoprotein, pharmacological treatment of hypertension, diabetes, and dyslipidemia, body mass index, smoking history, alcohol consumption, and metabolic syndrome.**

### E. Differences between agricultural and general workers with respect to the characteristics of oxidative stress markers

The mean age of the agricultural workers (age, 64.50 years) was higher than that of the general workers (age, 61.59 years) (Table 5). The percent of obesity measured by the BMI was higher in the general workers than in the agricultural workers ($P$ = 0.0139). The levels of oxidative stress markers, except for 8-OHdG, were higher in the agricultural workers than in the general workers (isoprostane, 0.39 [0.19–0.73] vs. 0.26 [0.15–0.46]; MDA, 0.16 [0.11–0.33] vs. 0.12 [0.07–0.18], median [interquartile range] in agricultural vs. general workers, respectively). There were no significant differences in the other atherosclerotic risk factors, such as the presence of MetS, alcohol consumption, smoking history, and pharmacological treatment of HTN, DM, and dyslipidemia. After adjustment for the pharmacological treatment of HTN, DM, and dyslipidemia, there was no significant change in the associations. There was a significant difference in the level of CIMT between the agricultural and general workers (1.00 ± 0.23 vs. 0.88 ± 0.14, respectively).

Table 5. Anthropometric and metabolic characteristics of the agricultural and general workers

| | Agriculture worker (n=63) | | General worker (n=39) | | p value | p value* |
|---|---|---|---|---|---|---|
| Age, year | 64.50 | ± 6.33 | 61.59 | ± 6.9 | 0.032 | |
| % of obesity, n (%) | | | | | | |
| Waist circumference ≥90cm | 15 | (23.81) | 15 | (38.46) | 0.114 | |
| Body mass index < 23 | 31 | (49.21) | 8 | (20.51) | | |
| Body mass index < 25 | 15 | (23.81) | 16 | (41.03) | | |
| Body mass index ≥ 25 | 17 | (26.98) | 15 | (38.46) | 0.014 | |
| % of alcohol drinking, n (%) | 47 | (74.60) | 22 | (56.41) | 0.056 | |
| % of never smoker, n (%) | 26 | (41.27) | 17 | (43.59) | 0.818 | |
| Presence of MetS, n (%) | 20 | (31.75) | 15 | (38.46) | 0.787 | |
| Pharmacologic treatment, n (%) | | | | | | |
| Hypertension | 26 | (41.27) | 13 | (33.33) | 0.423 | |
| Diabetes | 9 | (14.29) | 3 | (7.69) | 0.315 | |
| Dyslipidemia | 6 | (9.52) | 9 | (23.08) | 0.060 | |
| Isoprostane, ng/mg creatinine | 0.39 | (0.19-0.73) | 0.26 | (0.15-0.46) | 0.043 | 0.040 |
| 8-OHdG, μg/g creatinine | 1.07 | (0.62-1.81) | 0.78 | (0.52-1.31) | 0.086 | 0.074 |
| MDA, μmol/g creatinine | 0.16 | (0.11-0.33) | 0.12 | (0.07-0.18) | 0.003 | 0.003 |
| LDL, mg/dL | 114.50 | (93.00-132.00) | 114.00 | (90.00-140.00) | 0.994 | 0.992 |
| Triglyceride, mg/dL | 120.50 | (89.00-170.00) | 136.00 | (96-172) | 0.363 | 0.322 |
| CIMT, mm | 1.00 | ± 0.23 | 0.88 | ± 0.14 | 0.001 | 0.010 |

*_P_-value adjusted for the treatment of hypertension, diabetes, and dyslipidemia and age by rank transform ANCOVA.

Abbreviations: isoprostane, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; MDA, malondialdehyde; LDL, low-density lipoprotein cholesterol; MetS, metabolic syndrome; CIMT, carotid intima media thickness.

### F. Differences between the cases and controls in the case of agricultural workers with respect to the characteristics of risk factors

In the agricultural workers (Table 6), there were no significant differences between the cases and controls for age, obesity, alcohol consumption, smoking history, as well as the other metabolic risk factors such as triglycerides, LDL, presence of MetS, and pharmacological treatment of HTN, DM, and dyslipidemia. The levels of isoprostane and MDA, but not 8-OHdG, were higher in the cases than in the controls (isoprostane: 0.50 [0.30–0.72] vs. 0.19 [0.14–0.28]; MDA: 0.51 [0.34–0.65] vs. 0.41 [0.33–0.60], median [inter-quartile range] in cases vs. controls, respectively). The PEM was higher in the cases than in the controls (6.67 [0.10–13.33] vs. 4.00 [0.67–8.17], respectively, median [inter-quartile range]).

Table 6. Anthropometric and metabolic characteristics of the cases and controls in the case of agricultural workers

| | CIMT ≥0.9 (n=38) | | CIMT <0.9 (n=25) | | P value |
|---|---|---|---|---|---|
| Age, year | 63.76 | ±6.87 | 65.60 | ±5.39 | 0.2407 |
| % of obesity, n (%) | | | | | |
| Waist circumference, ≥90cm | 9 | (23.68) | 6 | (24.00) | 0.9770 |
| Body mass index < 23 | 17 | (44.74) | 14 | (56.00) | 0.4804 |
| Body mass index < 25 | 11 | (28.95) | 4 | (16.00) | |
| Body mass index ≥ 25 | 10 | (26.32) | 7 | (28.00) | |
| % of alcohol drinking, n (%) | 28 | (73.68) | 19 | (76.00) | 0.8363 |
| % of never smoker, n (%) | 12 | (31.58) | 14 | (56.00) | 0.0541 |
| Presence of MetS, n (%) | 13 | (34.21) | 7 | (28.00) | 0.6044 |
| Pesticide exposure month, month | 6.67 | (0.10-13.33) | 4.00 | (0.67-8.17) | 0.0221 |
| Pharmacologic treatment, n (%) | | | | | |
| Hypertension | 15 | (39.47) | 11 | (44.00) | 0.7211 |
| Diabetes | 7 | (18.42) | 2 | (8.00) | 0.2475 |
| Dyslipidemia | 2 | (5.26) | 4 | (16.00) | 0.2038 |
| Isoprostane, ng/mg creatinine | 0.50 | (0.30-0.72) | 0.19 | (0.14-0.28) | <.0001 |
| MDA, μmol/g creatinine | 0.51 | (0.34-0.65) | 0.41 | (0.33-0.60) | 0.0096 |
| 8-OHdG, μg/g creatinine | 1.12 | (0.64-1.81) | 0.76 | (0.44-1.07) | 0.2036 |
| LDL, mg/dL | 119.00 | (99-131) | 106.00 | (82-132) | 0.1111 |

Abbreviations: LDL, low-density lipoprotein; isoprostane, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; MDA, malondialdehyde; LDL, low-density lipoprotein cholesterol; MetS, metabolic syndrome.

### G. Logistic regression analysis between the pesticide exposure group and general workers

We compared the odds of developing subclinical atherosclerosis in the low or high pesticide exposure groups with that in the general workers (Table 7). The high pesticide exposure group had a higher risk of developing subclinical atherosclerosis than the general workers, but there was no significant difference between the odds of the low pesticide exposure group and the general workers. After adjustment for age, LDL, pharmacological treatment of HTN, DM, and dyslipidemia, BMI, smoking history, alcohol consumption, and MetS in model II, the high pesticide exposure group was at a higher risk of developing subclinical atherosclerosis (OR [95% CI]: 4.83 [1.56–14.95]). Further adjustments for oxidative stress markers attenuated this relationship, and the OR of the high pesticide exposure group lost its statistical significance (OR [95% CI]: 2.69 [0.75–9.73]).

Table 7. Relationships between occupational history of lifetime exposure to pesticides and risk of subclinical atherosclerosis

| Occupational history | Pesticide exposure | Model I | | Model II | | Model III | |
|---|---|---|---|---|---|---|---|
| | | OR | (95%CI)* | OR | (95%CI)* | OR | (95%CI)* |
| General worker | reference | 1.00 | - | 1.00 | - | 1.00 | - |
| Agriculture worker, pesticide exposure (dichotomized by median level) | low exposure (≤6 months) | 1.65 | (0.61-4.41) | 1.59 | (0.51-5.03) | 1.07 | (0.29-3.95) |
| | high exposure (>6 months) | 4.44 | (1.61-12.27) | 4.83 | (1.56-14.95) | 2.69 | (0.75-9.73) |



*OR (95% CI), odds ratio (95% confidence interval).

Model I: adjusted for age.

Model II: adjusted for age, low-density lipoprotein, pharmacological treatment of hypertension, diabetes, and dyslipidemia, body mass index, smoking history, alcohol consumption, and metabolic syndrome.

Model III: Model II plus adjustment for the oxidative stress markers 8-iso-prostaglandin F2α, 8-hydroxy-2'-deoxyguanosine, and malondialdehyde.

## H. Linear relationship between lifetime pesticide exposure and oxidative stress or subclinical atherosclerosis

We investigated the relationships between pesticide exposure and oxidative stress in agricultural workers (Figure 2). In the univariate regression model, there were significant relationships between PEM and oxidative stress (regression coefficient [B] = 0.0177, *P* = 0.0026 for isoprostane; B = 0.0077, *P* = 0.0028 for MDA; B = 0.0324, *P* = 0.0489 for 8-OHdG). Further adjustments for age, BMI, LDL, adiponectin, alcohol consumption, smoking history, and the presence of MetS strengthened these relationships for isoprostane and MDA (B = 0.0192, *P* = 0.0016 for isoprostane; B = 0.0085, *P* = 0.0027 for MDA), but not for 8-OHdG (B = 0.0300, *P* = 0.0857) (data not shown in the figure). There was a significant relationship between PEM and CIMT (B = 0.010, *P* = 0.0107).

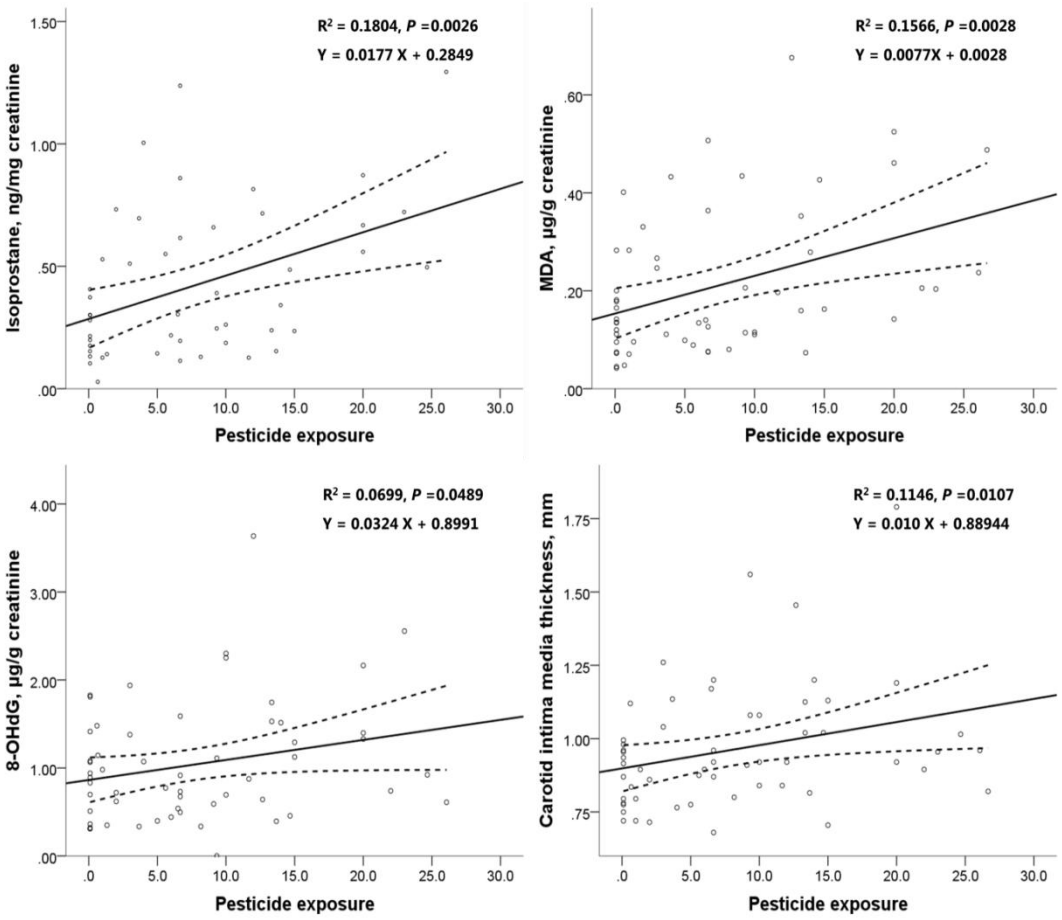


**Fig. 2. Scatter diagram between pesticide exposure (lifetime months of pesticide exposure) and oxidative stress or carotid intima media thickness in agricultural workers, showing the regression line (solid line) and 95% confidence interval (dotted lines)**

## I. Path analysis of the series connection between the lifetime exposure to pesticides and oxidative stress in subclinical atherosclerosis

We investigated the relationships between oxidative stress and pesticide exposure in subclinical atherosclerosis by path analysis. We used linear regression models to estimate the direct effect of oxidative stress and PEM in subclinical atherosclerosis. The backward selection method was used to select the risk variables for model fitting; therefore, 2 models, i.e., isoprostane with 8-OHdG and MDA with 8-OHdG, were used in Figure 2. In the univariate regression model, there was a significant linear relationship between subclinical atherosclerosis and PEM ($\beta = 0.357$, $P = 0.011$, data not shown in Figure 2). In the multivariate regression models, after adjusting for oxidative stress markers, there was no significant direct effect of PEM on subclinical atherosclerosis ($\beta = 0.146$, $P = 0.219$, in model A; $\beta = 0.161$, $P = 0.173$ in model B). We tested 2 models, including a hypothesized path between PEM, oxidative stress, and subclinical atherosclerosis. The model fit indices indicated the correlation of PEM with elevated levels of oxidative stress, and the elevated levels of oxidative stress were also correlated with an increased risk of developing subclinical atherosclerosis (chi-square = 1.449, degrees of freedom = 2, $P = 0.485$, CFI = 1.000, RMSEA < 0.0001 in model A; chi-square = 1.830, degrees of freedom = 2, $P = 0.401$, CFI = 1.000, RMSEA < 0.0001 in model B). All oxidative stress markers were

significantly associated with PEM (isoprostane: $\beta = 0.425$, $P < 0.01$; MDA: $\beta = 0.396$, $P < 0.01$; 8-OHdG: $\beta = 0.264$, $P < 0.05$). The oxidized lipid damage markers isoprostane and MDA were associated with CIMT, independent of 8-OHdG, the oxidized DNA damage marker, in both models (isoprostane: $\beta = 0.248$, $P < 0.01$ and 8-OHdG: $\beta = 0.328$, $P < 0.01$ in Model A; MDA: $\beta = 0.227$, $P < 0.01$ and 8-OHdG: $\beta = 0.330$, $P < 0.01$ in Model B).

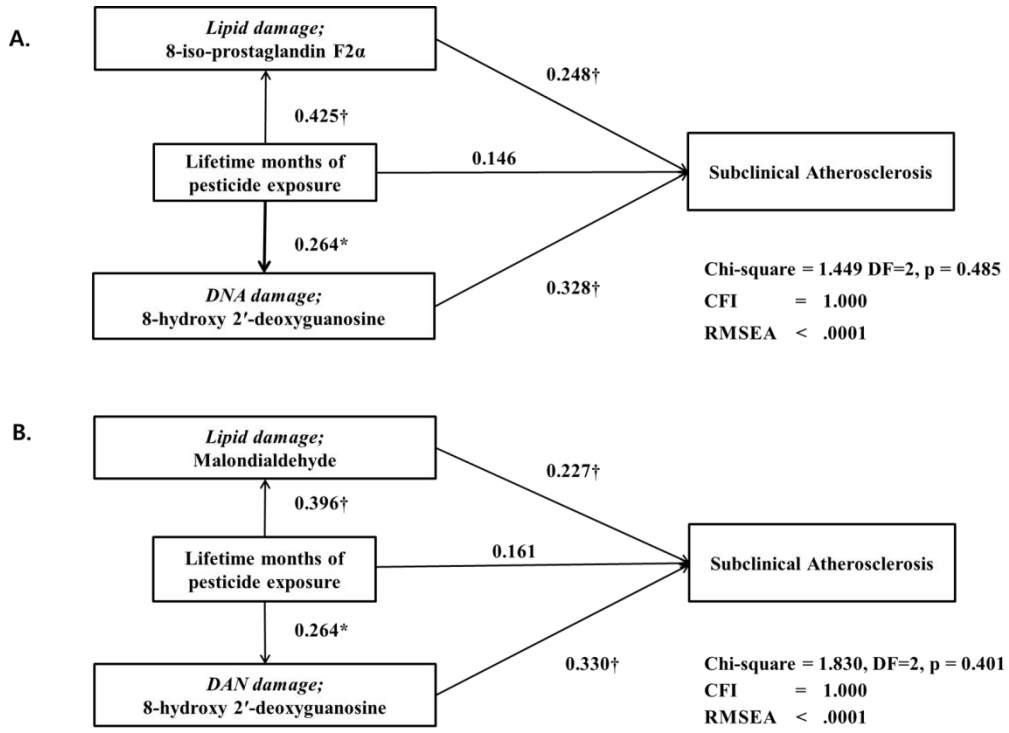


**Fig. 3. Results of the path analysis for the relationships between oxidative stress and pesticide exposure in subclinical atherosclerosis**

**The oxidative stress markers for lipid damage were 8-iso-prostaglandin F2α in (A) and malondialdehyde in (B). The standardized coefficients are listed on the model paths. The model fit indices are listed. CFI, comparative fit index; RMSEA, root mean square error of approximation; *$P < 0.05$, †$P < 0.01$.**

## IV. Discussion

In this nested case control study, we found that the elevated risk of subclinical atherosclerosis in agricultural workers was associated with increased levels of oxidative stress, which were related to pesticide exposure. The first results for the relationships between oxidative stress and subclinical atherosclerosis were generally consistent with other studies [18–20]. In addition, the second results for the association between pesticide exposure and oxidative stress were also generally consistent with other studies [11–13]. Furthermore, to the best our knowledge, we describe the first application of path analysis to elucidate the role of oxidative stress associated with pesticide exposure and subclinical atherosclerosis in agricultural workers.

The ORs of subclinical atherosclerosis were higher in agricultural workers than in general workers (Table 3). By comparing the occupational subgroups shown in Table 5, older age and higher levels of oxidative stress markers were found in agricultural workers than in general workers. Conversely, obesity parameters, which are strong risk factors for cardiovascular disease [21], were lower in the agricultural workers than in the general workers. After adjustments for conventional cardiovascular risk factors, including obesity parameters, a history of agricultural work was still significantly associated with subclinical atherosclerosis. Further adjustments for isoprostane or MDA attenuated these

associations, and a history of agricultural work lost its significant association to subclinical atherosclerosis. Therefore, a history of agricultural work was associated with subclinical atherosclerosis independently of age, obesity parameters, and conventional cardiovascular risk factors, such as MetS, but not with oxidative stress.

To investigate the interactive relationships, further analyses were undertaken using the 2 × 2 strata created on the basis of occupational subgroups and higher or lower levels of oxidative stress. There were interesting relationships between oxidative stress and the risk of subclinical atherosclerosis according to the occupational subgroups. The ORs of subclinical atherosclerosis in relation to oxidative stress levels were sharply increased in agricultural workers, more than the expected sum of the ORs. When comparing the observed and expected joint effects in strata analysis, occupational history and isoprostane showed an additive interaction with a synergistic effect on the risk of developing subclinical atherosclerosis. This synergistic effect between occupational history and oxidative stress highlights the relatively important role of oxidative stress on the risk of developing subclinical atherosclerosis in agricultural workers. This synergistic relationship remained significantly after adjustments for MetS and other conventional risk factors (Figure 1). Therefore, occupational history and its interaction with oxidative stress have a significant clinical meaning beyond the individual risk factors at the population level.

In Table 5, we tried to explain the different characteristics and to identify the variables that caused the differences in the risk of developing subclinical atherosclerosis between agricultural and general workers. Age and oxidative stress markers were the only reasonable risk factors that increased the risk of agricultural workers to develop subclinical atherosclerosis. There were no significant differences between the groups for the traditional cardiovascular disease risk factors, e.g., waist circumference, alcohol consumption, LDL, and triglyceride. Furthermore, BMI was lower in the agricultural workers. In Table 6, we compared the different characteristics of the risk factors between the cases and controls only for agricultural workers. There were significant differences between the cases and controls for isoprostane, MDA, and PEM, but not for age. Therefore, in the present study, oxidative stress makers were important risk factors that were significantly different across the general worker and agricultural worker cohorts, and between the case and control groups in the agricultural workers.

The results showing high levels of oxidative stress in agricultural workers were not fully analyzed in this nested case control study. We conducted further analysis of pesticide exposure and its association with oxidative stress and subclinical atherosclerosis. Exposure assessment of pesticides was conducted using PEM. PEM has previously been used to reveal the significant relationship between oxidative stress-related chronic disease and the incidence of cancer [14,22]. In the present study, we also used PEM to identify the high or low pesticide exposure groups in agricultural workers. The multivariate adjusted OR

in the high pesticide exposure group was higher than in the general workers; however, further adjustments for oxidative stress attenuated this association (Table 7). This attenuation suggested that the association between pesticide exposure and subclinical atherosclerosis was not independent of oxidative stress. Furthermore, PEM was correlated with oxidative stress markers (Figure 2) and CIMT. These results suggest that there could be multiple causal agents; therefore, additional advanced analyses, such as path analysis, are needed to provide a better explanation than the multiple regression models [23].

We performed path analysis. As shown in Figure 2, there were significant linear relationships between oxidative stress, PEM, and CIMT. During the repeated construction of multiple regression models using backward selection, we fitted 2 models. First, the oxidative stress markers of lipid damage, i.e., isoprostane and MDA, and DNA damage, i.e., 8-OHdG, were independently related to CIMT. We hypothesized that PEM exerted direct and indirect effects in subclinical atherosclerosis through oxidative stress, and the model fit indices supported our hypothesis (Figure 2). However, we did not observe any significant direct effects between PEM and CIMT in these 2 models.

Our results highlight the observation that agricultural workers are at an increased risk of developing subclinical atherosclerosis. Contrary to our results, the well-known Agricultural Health Study (AHS) showed lower risks of cardiovascular disease, DM, chronic obstructive pulmonary disease, and cancer mortality in agricultural workers when they were compared with the general

population [24]. Five years later, some of these deficits were considered to be the result of a “healthy worker effect” [25]. The healthy worker effect is a bias that can mask the risk of mortality or morbidity when an exposure group and general population are compared [26]. The selection of healthy workers can occur by the exclusion of unhealthy workers from the workplace [26], where healthy workers are continually employed, while workers who develop disease leave their workplace [27]. In some studies, the prevalence of cardiovascular disease in agricultural workers is lower than that observed in other workers. When comparing agricultural workers and construction workers, the prevalence of cardiovascular disease is higher in agricultural workers than in construction workers [28]. This suggested that the healthy worker effect might be minimized due to the same requirements of physical labor in agricultural and construction workers. To minimize the healthy worker effect, we tried to include not only active workers, but also included those who were no longer working [26]. When the participants were unemployed, we used their longest occupation as their occupational history and this may have minimized the healthy worker effect.

Oxidative stress has been linked with the toxicity of various pesticides such as organophosphate, organochlorine, carbamate pesticides, and synthetic pyrethroids [11,29]. Organophosphate and carbamate compounds inhibit the activity of acetylcholinesterase enzyme (AChE). Reduced AChE activity causes the accumulation of acetylcholine in the autonomic nervous system and adrenal medulla [30], which leads to excessive cholinergic signs and symptoms.

Organochlorine pesticides cause repetitive firing of action potentials by permanently opening sodium channels in neurons [31]. Type I and type II pyrethroids alter the function of sodium channels in neuronal membranes, and they are linked with the peripheral and central nervous systems, respectively [32]. However, the inhibition of AChE by itself or persistent sodium channel opening cannot account for the wide range of disorders that are linked to pesticide poisoning and various types of cell injury [33]. The lipophilic characteristics of pesticides enable their penetration into the cell membrane, potentially inducing oxidative stress [32,34]. A biochemical interaction study indicated that the mixed toxicity of organophosphates may not be essential for the AChE action pathway, but may be additive to the previous oxidative damage of other organophosphates [34]. Therefore, pesticides and coexisting chemicals may induce oxidative damage by generating ROS and inhibiting the antioxidant system [35]. If repeated exposure to pesticides has occurred, ROS attack biological macromolecules and cause oxidative damage when they cannot be removed by the physiological antioxidant system, and this damage accumulates and causes adverse health effects [11]. Oxidative stress markers and antioxidant enzymes were suggested as good surrogate biomarkers for pesticide toxicity [13]. These studies support our observation that oxidative stress, which increased atherogenic risk, could be a good surrogate biomarker for pesticide toxicity.

According to the oxidative modification hypothesis for atherosclerosis, the native state of LDL is not atherogenic [36]. When LDL becomes entrapped in the

sub-endothelial space, the apolipoprotein B-100 lysine groups on the surface of LDL are modified from its net charge. These oxidized modifications of LDL stimulate monocyte chemotaxis and inflammation [37]. Oxidized LDL is susceptible to uptake via the macrophage scavenger system, and this incorporation and accumulation into macrophages is a major cause of foam cell transformation and plaque formation [38]. In these complex steps, free radicals have a critical role and oxidize LDL lipids. These free radical attacks initiate lipid peroxidation, i.e., the oxidative degradation of lipids. Increased lipid peroxidation has been identified as a key mechanism for the development of atherosclerosis and inflammatory vascular damage. We used isoprostane and MDA as markers for oxidative stress in the form of lipid peroxidation.

Isoprostane, the stable isomer of prostaglandin F2α, is formed by the non-enzymatic free radical attack of arachidonic acid, which is a component of the lipid cell membrane. Some studies compared the daily variation in the levels of isoprostane in spot urine and 24-h urine samples, and observed no significant differences throughout the day in either samples [39]. The mean levels of isoprostane in morning urine samples were not significantly different from 24-h collection samples [40]. Therefore, the level of isoprostane in the urine is widely used as a gold standard marker for lipid peroxidation [41]. We also used HPLC-MS/MS, which has been reported to be one of the most reliable methods [42]. MDA is produced during the oxidative attack of lipoproteins and polyunsaturated fatty acids. The 2 oxidative stress markers of lipid peroxidation used in this study

were well correlated (Table 2). Therefore, MDA and isoprostane levels in urine are recommended as good surrogate markers of oxidative stress to assess the risk of developing subclinical atherosclerosis according to the results presented in the current study.

The 8$^{th}$ position of guanine oxidative hydroxylation is the most mutagenic lesion [20], and its modified product is 8-OHdG. Elevated levels of 8-OHdG are associated with various malignant conditions [43] and their prognosis [44]. Apoptosis, i.e., programmed cell death, is induced by DNA damage [45]. Endothelial cell death is an early event of atherogenesis and triggers plaque formation [45]. Apoptosis of vascular smooth muscle cells (VSMCs) is associated with the growth of plaques by outward remodeling [46], and it triggers intense intimal inflammation [47], which induces foam cell formation by the accumulation of lymphocytes. Atherogenic lesions may be initiated in VSMC by mutational events, such as tumor cell growth through DNA damage [48]. Therefore, 8-OHdG is associated with atherosclerosis and plaque formation [20].

The oxidative stress markers examined in the present study were not associated with the obesity parameter BMI (Table 2). These results were in disagreement with those from the recent Framingham study [49] that highlighted the relationship between obesity and isoprostane levels. However, the BMI levels in our study (23.75 and 24.93 in the agricultural and general workers, respectively) were relatively lower than in the Framingham study [49] (28.8 in men). In the Framingham study, there was no significant correlation between the levels of

isoprostane and BMI until the BMI reached 29.1 [49]. Furthermore, obesity and oxidative stress showed an inverse correlation for a BMI < 29.1, which was the level observed in the participants of the present study. Therefore, the exact relationship between oxidative stress and obesity requires further analysis, and our results suggest that oxidative stress parameters are good surrogate markers of subclinical atherosclerosis, even in a non-obese population.

Several limitations were considered when interpreting the current results. The main limitation was the cross-sectional nature of our case control study design, from which a cause-effect relationship cannot be concluded. Furthermore, atherosclerotic lesions themselves produce oxidative stress, and this vicious condition was represented via the elevation of oxidative stress markers [37]. To minimize this effect, we excluded participants who had a cardiovascular event, chronic hepatitis, osteoporosis, kidney disease, asthma, or any malignant disease, but we could not exclude patients with HTN, DM, and dyslipidemia. Glycemic disorders induce oxidative stress, and a chronic hyperglycemic status, such as DM, is deeply associated with endothelial dysfunction, i.e., vascular disease [50]. We controlled for the effect of DM, HTN, and dyslipidemia using statistical methods, i.e., multiple regression models with limitations. Therefore, a prospective cohort study to elucidate the causal relationship between oxidative stress and atherosclerosis is warranted. Another limitation was due to the effect of drugs that alter the levels of oxidative stress. Rosuvastatin and biguanide, which are widely used for the treatment of dyslipidemia and DM, also decrease inflammation and

oxidative stress [51]. An angiotensin II receptor blocker also reduced blood pressure and oxidative stress [52]. These types of drugs were not specified according to their anti-oxidant effect in the present study. However, there were no significant differences of presence of pharmacological treatment between the case and control groups, although drug-specific effects cannot be eliminated. We have no information about the consumption of dietary vitamin supplements in our cohort, which could also reduce the levels of oxidative stress markers [53]. The use of dietary supplements could differ between occupational groups; for this reason, the high levels of oxidative stress in the agricultural worker group warrant a more detailed assessment of their life style patterns. To assess the exposure to pesticides, we used PEM, which is a simple method. The intensity weighted exposure day could be estimated with a detailed variable such as the use of personal protective equipment, pesticide application methods, pesticide mixing status, and equipment repair methods [22]. Furthermore, we used the questionnaires of pesticide application time, not pesticide exposure time, to estimate exposure assessment. Therefore, we couldn’t specify the pesticide exposure. However, both methods revealed a significant association with clinical disease in a previous study [22]. Because of the relative small sample size of the current study, and the fact that the samples were chosen from only 1 geographic area, our results cannot be generalized for all agricultural workers in Korea.

In summary, we observed an increased risk of subclinical atherosclerosis in agricultural workers who had higher levels of oxidative stress than general

workers. In addition, these higher levels of oxidative stress were correlated with pesticide exposure. The deducible pathologic links between agricultural workers and their high risk of developing subclinical atherosclerosis could be explained by the indirect effect of pesticide exposure through increased levels of oxidative stress. Therefore, we highlighted importance of assessing occupational history and oxidative stress to investigate the risk of developing subclinical atherosclerosis.

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# ABSTRACT (in KOREAN)

## 농업인에서 무증상 죽상경화증과 산화손상의 관계

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**배경**

심혈관계 질환의 복잡한 병태생리 메커니즘을 설명하기 위한 많은 연구들에서, 산화손상과 동맥경화 사이의 관련성은 강조되고 있다. 본 연구의 목적은 농업인에서 무증상 죽상경화증과 산화손상의 관계를 조사하는 것이다.

**대상 및 방법**

코호트 내 환자대조군 연구로, 2011 년 5 월부터 2011 년 8 월까지의 방문자중, 뇌졸중, 뇌출혈, 심근경색, 협심증 및 악성종양이 없는 사람들을 대상으로 하였다. 이들 중, 경동맥 내중막 두께 (carotid intima media thickness, CIMT)가 0.9 mm 이상인 51 명을 환자군으로 선정하였다. 연령에 따른 빈도대응추출법을 통해, CIMT <0.9mm 인 51 명을 대조군으로 선정하였다. 산화손상 지표로는 8-hydroxy 2′-deoxyguanosine (8-OHdG), malondialdehyde (MDA), 8-iso-prostaglandin F2$\alpha$ (Isoprostane)를 측정하였다. 농약 노출에 대해서는 농약노출기간 (lifetime months of pesticide exposure)을 설문 내용을 토대로 산출하였다. 조건부 로지스틱 회귀분석을 이용하여, 무증상 죽상경화증 발생 위험에 대한 직업력과 산화손상 수치의 상대적 관련성을 분석하였다.

**결과**

비농업 및 낮은 산화손상군으로 이루워진 집단에 대하여, 농업인 및 높은 산화손상 군은 무증상 동맥경화증에 대한 높은 비교위험도(Odds ratio, OR)를 보였다 (OR [95% Confidence interval], Isoprostane, 16.00 [3.99-64.18]; MDA, 10.40 [3.07-35.28]; 8-OHdG, 8.64 [2.57-29.07]). 농업인에 대해서만 추가로 진행된 경로 분석에 대해서는 농약 노출은 산화손상을 증가시키고 ($\beta$=0.425 [Isoprostane], $\beta$= 0.396 [MDA], $\beta$= 0.396 [8-OHdG]), 증가된 산화손상은 CIMT 와 양의 상관관계를 보였다 ($\beta$= 0.248 [Isoprostane], $\beta$= 0.227 [MDA], $\beta$= 0.330 [8-OHdG]).

**결론**

농업인은 비농업인에 비해 산화손상 지표의 수치 증가와 무증상 죽상경화증의 발생 위험이 높았다. 증가된 산화손상 수치는 농약 노출과 양의 상관관계가 있었다. 농약 노출에 의한 산화손상 수치의 상승은 농업인에서 무증상 죽상경화증의 발생 위험도를 높였다.

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주제어(Key words): 산화손상, 농업인, 무증상 죽상경화증, 동맥경화.