Decreased Antioxidant Capacity in Corn Farmers Occupationally Exposed to the Mixture of Herbicides

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Abstract

Background: The application of herbicides has been a common practice in worldwide agriculture, mainly with the goal to increase efficacy of weed control. So, most farmers faced a greater risk of herbicide exposure to develop adverse health effects from oxidative stress-induced herbicides. The aim of this study is to determine the urinary total antioxidant capacity (TAC) and 8-isoprostane levels in farmers using the mixture of herbicides in Long District, Phrae Province, Thailand.

Methods: Ninety-three participants were recruited. The spot urine samples (pre- and post-work) were collected. The urinary TAC was determined using ABTS radical scavenging assay. The urinary 8-isoprostane level was investigated by commercial ELISA kit.

Results: Most farmers worked on a farm during 1-5 h/day. Fifty-five percents of participants used the mixture of herbicide while working between glyphosate and paraquat as well as glyphosate and 2,4-D. The urinary TAC levels in pre-work urine sample of farmers applied combining herbicides were significantly higher than that level in their post-work urine sample. However, there was no significant difference between pre- and post-work urinary 8-isoprostane in farmers applied combining herbicides.

Conclusion: The results suggest that occupational exposure to mixture of glyphosate herbicides plus paraquat or 2,4-D could induce abnormal oxidative stress value especially antioxidant defense among agricultural workers.

Keywords: *8-isoprotane, Glyphosate, Paraquat, Total antioxidant capacity (TAC)*

Introduction

Herbicides are being widely used for control the growing of weeds in agricultural activities[1]. Although herbicides are totally beneficial for agricultural activity, they are crucial sources of environmental pollution and exert irreversible adverse health outcomes both short- and long-term effects. The exposure of herbicides in workers generally caused from unintentional direct consumption, direct contact to skin due to improper application, inhalation of aerial sprays, or contaminated food consumption[2]. In the case of Thai farmers, there has been reported to have the high risk for herbicide exposure during occupation since most farmers practically apply high volume of the herbicide mixtures than recommended dosages for increase capability of weed control. As a result, these farmers faced greater risk of exposure to herbicides and development of adverse health effects[3]. The imbalance between oxidant and antioxidant defenses known as oxidative stress has been proposed as a key player in accounting for

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herbicide toxic effect[4, 5]. The overproduction of oxidants especially reactive oxygen species (ROS) caused adverse modifications to cell components such as lipids, proteins, and DNA damage. Lipid peroxidation is a one process of cell membrane-attacked ROS[6]. The by-products of this process such as malondialdehyde (MDA), propanal, hexanal, and 8-isoprostane were released and detected into various biological fluids[7]. In addition, the excess ROS was scavenged by effective protective mechanisms using antioxidant substances for preventing the attack of ROS on biomolecules and cells[8].

Previous studies demonstrated that the increase of lipid peroxidation by-product (8-isoprostane) was induced in corn farmers exposed with mixtures of herbicide usage between atrazine and 2-(2,4-dichlorophenoxy) acetic acid (2,4-D)[9, 10]. Available studies about antioxidant defense has been reported that superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) level was significantly decreased in herbicide-exposed workers[11, 12]. Hence, the investigation of oxidative stress status in farmers who occupationally exposed with mixture of herbicides need to be focused for health status evaluation. In the present study, we hypothesized that the imbalance between reactive substance in forms of 8-isoprostane and antioxidant activity could be induced by mixture of herbicides (glyphosate or paraquat or 2,4-D). The main objective of this study was to determine the urinary 8-isoprotane and TAC levels in farmers using of the groups of herbicides during agricultural activities in Long District, Phrae Province.

Material and Methods

Ethical approval

This study was approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (Study code: FOR-2562-06349). All subjects were informed about the protocol of this study and signed the consent form according to the guidelines of the Faculty of Medicine ethical committee.

Location and population

This study was conducted in Long district, Phrae province, Thailand. Ninety-three participants were eligible for this study. The inclusion criteria consisted of 1) farmers aged between 30 to 60 years 2) have been using combination of glyphosate or paraquat or 2,4-D during the study period 3) have never been diagnosed with kidney disease, diabetes and gout. All individuals were interviewed face-to-face using a questionnaire documenting their sex, working hours on the farm, year of work, and type of herbicide usage. All participants were divided into two groups based on the type of herbicide use 1) single application; farmers who applied only glyphosate 2) combined application; farmers who applied the mixture of herbicides in the group of glyphosate or paraquat or 2,4-D.

Urine sample collections

The spot morning urine before work (pre-work) and next morning urine after working (post-work) were collected from each participant. All samples were stored at −20 °C prior the quantification of urinary creatinine, 8-isoprostane, and total antioxidant capacity (TAC)[13].

Quantification of urinary creatinine levels

The urinary creatinine level was determined by colorimetric assay of Jaffé method[14]. In brief, 50 µL of urine samples (dilution 1:50) was mixed with 200 µL of working solution (1:1 of 4.365 mM picric acid (Sigma-Aldrich, USA) and 0.25 M sodium hydroxide (Sigma-Aldrich, USA)). After 45 min of reaction times, the absorbance of the orange-red creatinine picrate reaction product was measured at a wavelength of 492 nm using microplate reader (Synergy™ H4, BioTek Instruments, Inc., USA). The level of urinary creatinine was calculated by comparing with creatinine standard curve and expressed as mg/dL.

Quantification of urinary 8-isoprotane levels

The urinary 8-isoprotane were measured using commercial ELISA kit (Abcam, UK) according to the manufacturer's instructions. The assay is based on the competitive ELISA between 8-isoprostane in sample and reference for binding to the primary antibody. The level

of urinary 8-isoprostane was calculated by comparing with 8-isoprostane standard curve and expressed as ng/mg creatinine.

Quantification of urinary total antioxidant capacity (TAC) levels

The urinary TAC was determined using ABTS radical scavenging assay[15]. Fresh $ABTS^{\bullet+}$ solution was prepared by the mixture between 1.0 mM of AAPH and 2.5 mM of ABTS dissolved in 100 mL of phosphate buffer solution (PBS), pH 7.4. The mixture was incubated at 70ºC for 30 min. Five microliters of urine sample was mixed with 245 µL of $ABTS^{\bullet+}$ solution and incubated at 37 º C for 10 min. The color reaction was measured at a wavelength of 734 nm by microplate reader (Synergy™ H4, BioTek Instruments, Inc., USA). The urinary TAC level was calculated by comparing with ascorbic acid (Biochemica, Scotland) standard curve and expressed as mg vitamin C equivalent/L (mg VCE/L).

Statistical Analysis

The statistical analysis was conducted using the SPSS for Windows (Version 16.0. Chicago, SPSS Inc; 2007) and the GraphPad Prism (version 8.3.0 for windows, GraphPad Software, San Diego, California USA, www.graphpad.com). All demographic data (gender, working hours in farm, year of work, and type of herbicide usage) were analyzed by descriptive statistics. The levels of 8-isoprostane and TAC were calculated by comparing between pre-work and post-work urine samples using the Wilcoxon signed-rank test.

Results

Ninety-three participants were randomly recruited from five villages in Thung Lang subdistrict, Long District, Phrae province. The characteristics of these participants are shown in Table 1. The majority of participants were male (62.36%). Sixty-two percent of farmers worked on a farm during 1-5 h/day. Most of the participants worked for at least 20-40 years (60.44). Approximately 55.91% of farmers used the single type of herbicides (glyphosate) and 44.09% of farmers used the mixture of glyphosate plus paraquat or 2,4-D.

To determine oxidative stress in subjects, the finding of urinary TAC in all participants is shown in Figure 1. The urinary TAC level in the pre-work and post-work urine samples of farmers who used single herbicide was ranged between 0.08 – 1.04 and 0.15 – 1.30 VCE/L, respectively. The urinary TAC level in the pre-work and post-work urine samples of farmers who used the mixture of herbicides was ranged between 0.10 – 1.42 and 0.10 – 1.28 VCE/L, respectively. The urinary TAC levels in pre-work urine sample of farmers used combination of herbicide were significantly higher than that level in the post-work urine sample. However, there were no significant differences of the urinary TAC levels between the pre-work and post-work sample in farmer who used single herbicide.

To determine the lipid peroxidation, the urinary 8-isoprostane was measured using commercial ELISA kit. The urinary 8-isoprostane level is shown in Figure 2. The urinary 8-isoprostane level in the pre-work and post-work urine samples of farmers who used single herbicide was ranged between 119.6 – 242508 and 80.02 – 249859 pg/mg creatinine, respectively. The urinary 8-isoprostane level in the pre-work and post-work urine samples of farmers who used the mixture of herbicides was ranged between 230.0 – 11164 and 168.6 – 4601 pg/mg creatinine, respectively. There was no significant difference between the urinary 8-isoprostane in pre-work and post-work sample of both groups (single and combination of herbicides use).

Table 1 Demographic characteristics of participants from studied area in Thung Lang subdistrict, Long District, Phrae province.

Characteristics	N	%
Gender		
male	58	62.36%
female	35	37.64%
working hours in farms		
1-5 hours/day	49	62.82%
6-12 hours/day	29	37.18%
year of work		
< 19 years	13	14.29%
20 – 40 years	55	60.44%
> 40 years	23	25.27%
Type of herbicides usage		
single	52	55.91%
combination	41	44.09%
Use of personal protective equipment		
Mask	90	96.77%
Glove	81	87.1%
Boots	92	99%

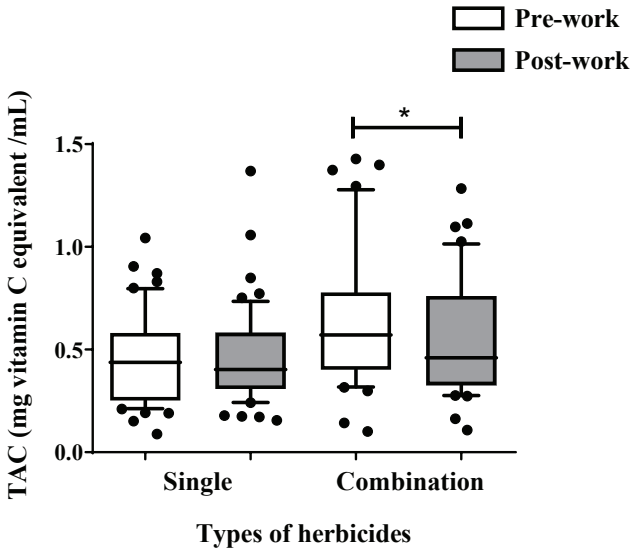

Figure 1 Box-plot demonstrating urinary TAC in herbicide-exposed farmers in the pre-work (open bar) and post-work (solid bar) urine sample. The line through the middle of the boxed represents the median of urinary TAC and the top and bottom of each box represents the first and third quartiles. The lower and upper error bars are computed as the lower and upper quartiles. *The TAC level was significantly different between the groups (P < 0.05).

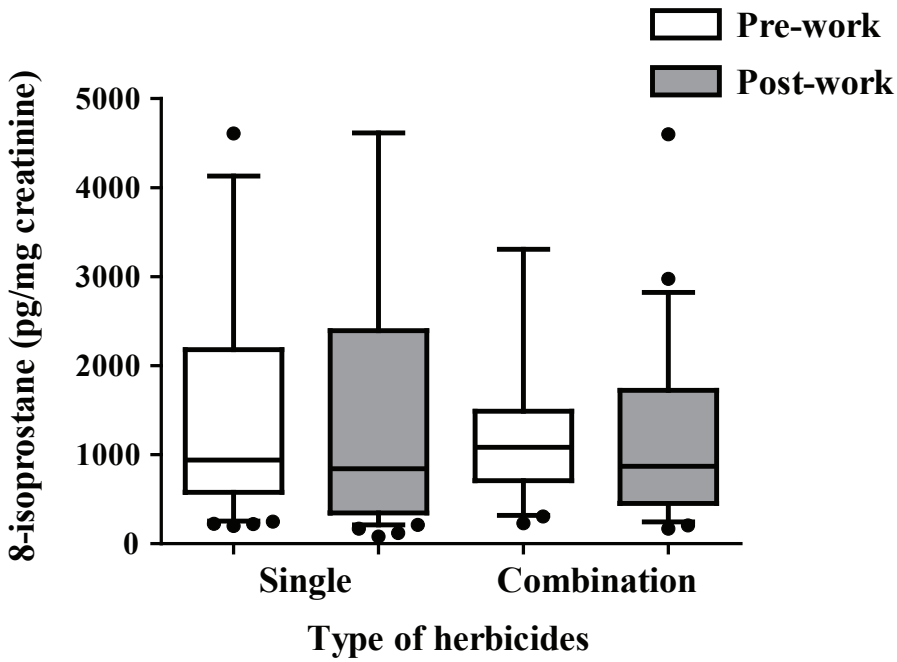

Figure 2 Box-plot demonstrating urinary 8-isoprostane in herbicide-exposed farmers in the pre-work (open bar) and post-work (solid bar) urine sample. The line through the middle of the boxed represents the median of urinary 8-isoprostane and the top and bottom of each box represents the first and third quartiles. The lower and upper error bars are computed as the lower and upper quartiles.

Discussion

Occupational exposure of herbicides has been reported to induce several health outcomes. The mixing two or more herbicides together was practically applied in the farms rather than the single use of herbicide. Previous report revealed that glyphosate herbicide was mostly used in a mixture with other herbicides to enhance its potency for weed control[16]. Therefore, it was probable that the farmers who applied the mixture of herbicides were at high risk of developing negative health effects resulting from oxidative stress-induced herbicides. The oxidative stress biomarker of lipid peroxidation, (8-isoprostane) and antioxidant defenses (TAC) indicating the imbalance of oxidative stress were determined this study. Our result found that the the urinary TAC levels in pre-work urine sample of farmers used combination of herbicides were significantly higher than that level in the post-work urine sample. The decrease of urinary TAC level could be indicative of oxidative stress or increased susceptibility to oxidative damage. Many classed of herbicides exerted the ability to generate reactive species, especially oxygen species molecules. Chronic oxidative stress could also reduce body antioxidant resources leading to lower antioxidant levels[17]. Moreover, the level of two antioxidant substances; superoxide dismutase (SOD) and glutathione reductase (GR) was decreased in pesticide sprayers[18]. Occupational exposure to pesticides among soybean farmers has been found to exert adverse effects at the biochemical level by observation of the alteration of antioxidant capacity in terms of ferric-reducing ability of plasma (FRAP) during exposure periods[19]. According to the study of Ojha et al. 2011[20] the level of antioxidant enzymes including catalase, superoxide dismutase, and glutathione peroxidase was decreased in dose-dependent in rats received the combined organophosphate pesticides.

Then, we aimed at assessing whether the decrease of antioxidant capacity from herbicide exposure may contribute to the elevation of oxidative stress marker. Previous studies reported that malondialdehyde (MDA) was the most popular marker for investigation of lipid peroxidation in vitro, however the level of MDA was a non-specific maker and depended on diet content[21-23]. An alternative approach was to determine 8-isoprostanes since it was stable, unaffected by lipid contents in a diet, high specific, and sensitivity products of peroxidation[7, 24]. The finding showed that there was no significant difference between the urinary 8-isoprostane in pre-work and post-work sample of both groups. There are two reasons for supporting our result. Firstly, the concentration of herbicides from occupational exposure may not be high sufficient to generate ROS, so the level of ROS may not be enough to disrupt lipid of cell membrane. Secondly, anioxidant defenses in the body can reduce ROS and maintain redox homeostasis resulting in unchange the level of 8-isoprostane[25, 26]. Thirdly, working time of farmers was less than 5 h/day and they wore personal protective equipment (PPE) during their working, they could therefore be exposed to low level of herbicides[27]. According to the study of Sapbamrer et al., 2019 the significant increase of SOD activity during the pesticide application season was associated with the number of working hours on the farm[28]. Moreover, the oxidative stress markers including MDA and superoxide dismutase were reported to have the significant correlation with number of worked hours/day, hours of spraying pesticides/day in pesticide-exposed agricultural workers[29].

Conclusion

In the light of present findings, it can be concluded that occupational exposure to mixture of herbicides between glyphosate or paraquat or 2,4-D could induce abnormal oxidative stress index especially antioxidant defense among agricultural workers. Our findings could be applied for health surveillance in farmers who had a high risk to occupational herbicide exposure.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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