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Serum Heat Shock Protein (HSP70) and Its Antibody (Anti-HSP70) Levels in Male Foundry Workers in Taiwan

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Heat shock proteins (HSPs) first observed in *Drosophila melanogaster* (Ritossa 1962) are very conservative proteins found from bacteria to mammals. HSPs are classified into several families according to their molecular weights including Hsp90, Hsp70, Hsp60 and those with Low Molecular Weights (LMW's) (Lindquist and Craig 1988). The levels of HSPs in cells can be elevated owing to environmental stresses such as extreme temperature, heavy metals, and organic compounds, and therefore are referred to as stress proteins as well. Because HSPs can be induced by many environmental stresses, HSPs, especially Hsp70 and Hsp60, have used as biomarkers in a range of algae, invertebrates, fish etc (Gibney et al 2001; Lewis et al 1999; Pyza et al 1997). The importance of HSPs in biochemical processes is their involvement in the folding of cellular proteins (Hartl 1996) thus ensuring the proteins perform normally. Induction of the HSPs by any environmental stress will make cells more resistant to environmental stress. The expression of HSPs has been extensively investigating by many researchers in the last decades (Craig et al 1993; Lindqust and Craig 1988; Minowada and Welch 1995).

The best-known HSPs are the sp70/Hsc70 family which is highly inducible. Overexpression of Hsp70 will prevent cells from being damaged by harmful stresses (Laszlo 1988). For instance, elevated levels of Hsp70 in heart and brain can transiently protect these organs from ischemic injury (Currie et al 1993; Plumier et al 1997). However, the production of antibodies against Hsp70 may cause some adverse health effects owing to occupational exposure (Wu et al 1998; Yang et al 2004; Yuan et al 2005). Foundry workers usually work in a harsh environment and suffer from many occupational stress factors such as high temperature, polyaromatic hydrocarbons, metal oxides, free silica, diphenyl methane diisocyanate and other organic matter (Palmer et al 1981). All these factors will induce the expression of Hsp70, and very few data regarding serum Hsp70 and anti-Hsp70 levels for foundry workers are available in Taiwan. Thus, this study tries to identify the presence and levels of serum Hsp70 and its antibody in foundry workers.

MATERIALS AND METHODS

A total of 87 male foundry workers participated to this study. The average age

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was 48.6 years old with a standard deviation of 9.8. Each worker was conducted with a regular medical examination, and additionally, a full-sized PA chest radiograph was also taken by an experienced radiographer using a mobile X-raymachine. Pneumoconiosis was diagnosed by a thoracic specialist. Each participator provided a self-filled questionnaire to indicate his personal information (i.e. name, age, gender etc.) as well as his personal behavior (i.e. smoking, alcohol and seafood consumption etc.). Venous blood was collected in heparinized tubes to separate plasma for the detection of Hsp70 and anti-Hsp70.

The commercial Hsp70 and anti-human Hsp70 ELISA (enzyme-linked immunosorbent assay) kits purchase from StressGen Biotechnologies (Victoria, BC, Canada) were employed to quantify Hsp70 and its antibody levels in sera from 87 foundry workers. The detailed experimental and calculation procedures can be obtained from the manufacturer's instructions (Stressgen, Victoria, BC, Canada). Briefly, the Hsp70 ELISA kit consisted of ELISA plates pre-coated with a mouse monoclonal antibody for inducible Hsp70 specifically. Inducible Hsp70 was trapped by the immobilized antibody and then detected with biotinylated rabbit polyclonal antibody. An avidin-horse-radish peroxidase conjugate was subsequently used to bind with the biotinylated detection antibody. The assay was then developed with tetramethylbenzidine (TMB) substrate and the color development was stopped by an acid stop solution and the intensity of the color was measured in a microplate reader at 450 nm. For anti-Hsp70 antibody, the ELISA plates pre-coated with recombinant human Hsp70 antigen were used to capture the antibody and then bound with an horseradish peroxidase conjugated polyclonal antibody specific for human IgA, IgG, IgM antibodies. The assay was also developed with TMB substrate and the color development was stopped by an acid stop solution. The intensity of the color was measured in a microplate reader at 450 nm, too. The standard Hsp70 protein and anti-human Hsp70 provided by StressGen Biototechnologies were used to generate the standard curves that were utilized to calculate the concentrations of Hsp70 and its antibody in sera. According to the manufacturer, the sensitivities of Hsp70 and anti-Hsp70 were < 0.5 and 6.79 ng/mL with the precision < 10%, respectively. This study utilized EXCEL (Microsoft EXCEL2003, Redmond, WA, USA) and SPSS package (Version 12.0; SPSS, Chicago, IL, USA) to conduct statistical analysis. Descriptive statistics including arithmetic mean (AM), standard deviation (SD), were calculated. The Kolmogorow- Smirnov's test was carried out to examine if the variables had a normal or a log-normal distribution. One-way analysis of variance (ANOVA) was employed to examine the hypotheses that serum Hsp70 and its antibody levels were significantly influenced by age and smoking behaviors. This model was also used to comparing the means of these workers with or without pneumoconiosis. A P <0.05 (two-tailed) was considered statistically significant for hypotheses test.

RESULTS AND DISCUSSION

The average serum concentrations of Hsp70 and anti-Hsp70 antibody are 2.85 ± 2.59 ng/mL and 188.9 ± 151.8 μg/mL, respectively. The HSP70 level ranges from

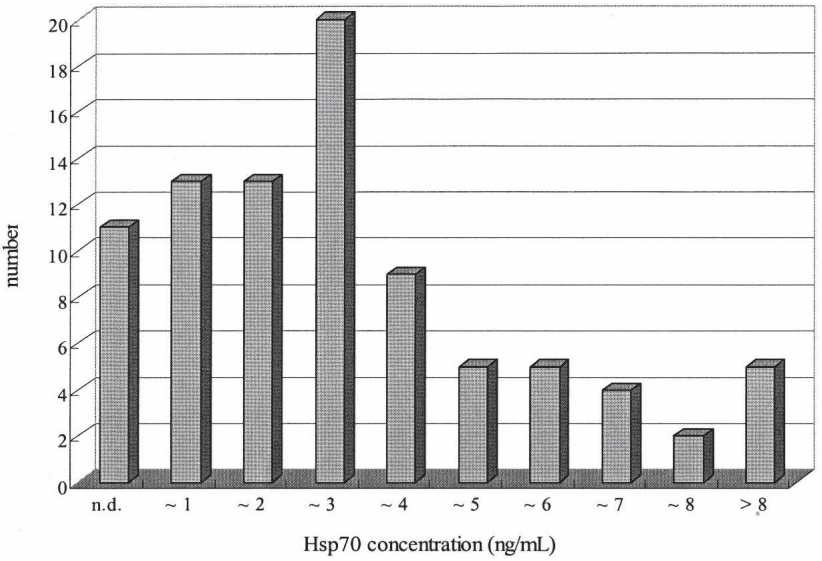

Figure 1. Distribution of serum Hsp70 levels.

0 (not detected, n = 11) to 10.63 ng/mL with a normal distribution (skewness: 1.22) while the anti-Hsp70 antibody level ranges from 27.5 to 948.8 μg/mL with a log-normal distribution (skewness: 0.179) (Figures 1 and 2). Among all participants, only 43 workers provided their current smoking status where 34 workers were smokers. The age distribution of these smokers (47.3 ± 9.8) is similar to the overall studied group. The average serum concentrations of Hsp70 and anti-Hsp70 antibody in these workers are 3.22 ± 2.96 ng/mL and 210.8 ± 186.5 μg/mL, respectively. There is no statistical difference on the serum Hsp70 and anti-Hsp70 antibody levels between smokers and non-smokers (Hsp70: 3.15 ± 3.44 ng/mL; anti-Hsp70: 156.8 ± 118.5 μg/mL). The age of subjects studied here ranges from 23 to 71; however, it's not randomly distributed owing to the casting industry is being phased out in Taiwan. In addition, the serum levels of Hsp70and its antibody are not significantly influenced by aging (Table 1); however, the antibody level seems to increase with age. The increase of Hsp70 and decrease of its antibody in sera of workers older than 60 years old may be as a result of "health worker effect" because most workers retire at age of 60. However, this suggestion needs be further investigated because of only few people studied here older than 60.

Fifty-three of all participators were diagnosed with pnumoconiosis. Their serum Hsp70 level averages at 2.80 ng/mL with a standard deviation of 2.38 ng/mL, which is not statistically different from the healthy workers. By contrast, their serum anti-Hsp70 antibody level (180.1 ± 24.8 μg/mL) is significantly higher than that of healthy workers ($p < 0.02$). It is noteworthy that these patients （51.3 ± 6.2 years old） were older than those healthy worker （43.9 ± 12.9 years old），

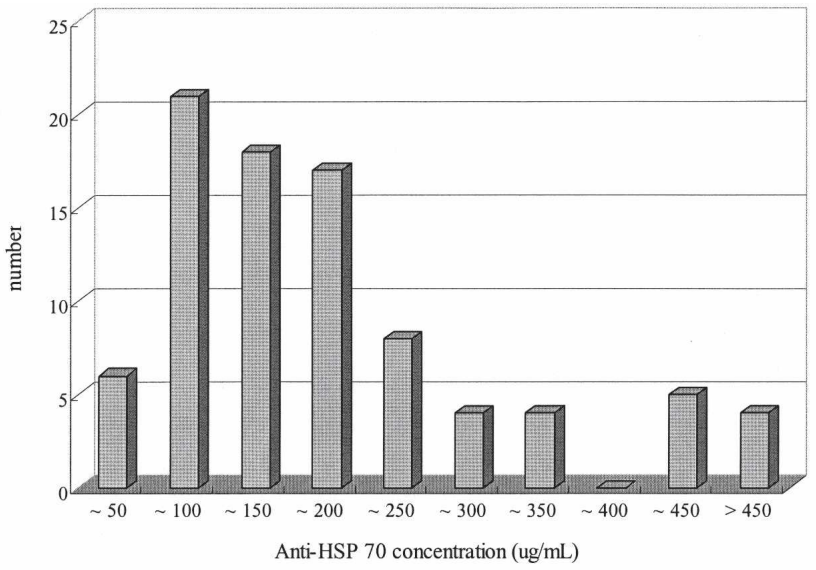

Figure 2. Distribution of serum anti-Hsp70 antibody levels.

although the aging was not observed significantly influencing on the serum Hsp70 and anti-Hsp70 antibody in our study here.

Table 1. Serum Hsp70 and anti-Hsp70 antibody levels in different age groups.

Age (year) (n)	Hsp70 (ng/mL)	Anti-Hsp70 antibody (μg/mL)
23 ~ 40 (12)	3.01 ± 2.65	160.5 ± 95.3
< 50 (31)	3.34 ± 2.80	191.6 ± 198.0
< 60 (38)	2.26 ± 2.10	201.8 ± 126.9
> 60 (4)	4.15 ± 4.37	126.3 ± 84.1

Compared with the reported serum Hsp70 levels of healthy populations (Jin et al 2004; Pockley et al 1998; Rea et al 2001), our observation is significantly lower. The phenomenon may be as a result of the utilization of the commercial ELISA kit (Njemini et al 2005). Additionally, our observation suggests that the serum Hsp70 levels would not significantly vary with age whereas a decline with age was observed by others (Jin et al 2004; Pockley et al 1998; Rea et al 2001), in particular, after age forty.

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