Plasma adipokine levels in Thais

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Summary

Objective: **To assess three plasma adipokines that might act as biomarkers useful in determining persons exposed to asbestos.**

Method: **Sixty subjects were included in the study, 30 male workers from factories manufacturing cement products using chrysotile asbestos, and 30 volunteer controls comprising 15 men and 15 women. Fasting venous blood specimens were submitted for blood chemistry examination and analysis of plasma levels of three adipokines, i.e. adipsin, adiponectin and resistin.**

Results: **(1) Compared with non-obese subjects, the adiponectin levels were lower in obese subjects, resistin levels were higher, and hemoglobin concentrations were lower. (2) Adipsin levels in the workers were significantly higher than in the controls ($p < 0.005$), and this difference was not related to body fat. (3) There were no statistically significant differences in adiponectin, resistin, and adipsin levels between the male and female controls. (4) Adiponectin in the male controls was significantly higher than that in the male workers ($p < 0.05$). (5) Female controls had significantly higher percentages of body fat ($p < 0.0005$) and resistin ($p < 0.02$) levels than male controls and male workers. (6) A significant negative correlation existed between resistin and hemoglobin levels levels ($r = -0.336$, $p < 0.01$). (7) Overall adipsin levels among male workers were significantly higher than among control subjects ($p < 0.005$); the six workers in whom asbestos bodies (AB) were detected did not have significantly higher levels of adipsin than those of workers without AB having been detected.**

Conclusions: **Although the findings apparently showed higher adipsin levels in the workers, its value as a biomarker for asbestos exposure requires confirmation from studies on a larger group of subjects. *(Asian Pac J Allergy Immunol 2015;33:59-64)***

Keywords: *adipsin, adiponectin, cement factory workers, chrysotile asbestos, resistin*

Introduction

In Thailand, there have been reports of a few patients diagnosed as having asbestos-related diseases who had a mere history of exposure risk to asbestos pollution but without sound evidence, except for an undiagnosed case of asbestosis concomitantly occurring in a case of talc pneumoconiosis,[1] while the diagnosis in other cases was merely circumstantial. In a previous study, workers with pulmonary disease who had radiographic evidence of asbestos–related pulmonary lesions that conformed with the standard classification of the International Labour Organization (standard ILO 2000 classification) had no pathological confirmation.[2] Recently, there have been reports of findings that certain cytokines, especially plasma adipokines, might be markers for asbestos-related disease in those in whom no asbestos materials had ever been detected in their bodies.[3,4]

Adipokines are cell proteins (cytokines), namely adiponectin, adipsin, leptin, and resistin, which derive from adipose tissue and sometimes also from leukocytes in response to inflammation. Logically, the same events would occur whenever humans are exposed to asbestos pollution, mostly by inhalation, or stimulation with intrusive asbestos fibers; thus, adipokines would be produced and released into the blood stream.

Leptin and resistin are potential pro-inflammatory cytokines, which play a role in attracting inflammatory cells in order to get rid of foreign bodies, while adiponectin is an anti-

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inflammatory cytokine that inhibits inflammation which would damage organs and tissues. On the other hand, although adipsin is also a pro-inflammatory cytokine, its main action is as a rate-limiting enzyme of the alternative complement cascade.

Reports from a number of research studies have indicted that adipokines initiate the onset of asthma and a few other yet unexplainable pulmonary diseases.[5,6]

Leivo-Korpela et al.[7] reported the results of studies of plasma adipsin occurring with pulmonary fibrosis among workers in industries using asbestos materials; the correlation of plasma adipsin levels with the occurrence of pulmonary fibrosis ($r = 0.412$, $p < 0.001$) and plasma adipsin in workers with pulmonary fibrosis was somewhat higher than those in people not exposed to asbestos.

The present study was undertaken with the aim of substantiating the possibility that the presence of any of the plasma adipokines would suggest definite asbestos invasion in persons suspected of having been exposed to asbestos, or would provide definite evidence of asbestos-related disease in patients who were suspected of having this disease.

Methods

The subjects included 30 men aged 24-65 years, who had worked in two cement factories using chrysotile asbestos for at least three years (25 of them had been working in such jobs for 21 to 35 years), and 15 men and 15 women volunteers aged 23 to 57 years from the general population who had no history of asbestos exposure. All 60 subjects informed the researchers of their health status.

10 milliliters of venous blood were collected from all subjects in the morning after a fast of 8-12 hours. A 7 mL aliquot was submitted for the determination of blood chemistry, i.e hemoglobin concentration, blood sugar, serum cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, and uric acid, using an automated blood BS-400 Chemistry Analyser, Mindray Bio-Medical Electronics Co., Ltd.[8]

Plasma from 3 mL of EDTA blood was analyzed for the presence of adipsin, adiponetin, and resistin using a Milliplex MAP, Merck Millipore, Germany.[9]

Body composition, i.e. body weight, body mass index, percentage of body fat, bone mass, muscle mass, and visceral fat, was assessed in the 60 subjects using TANITA BC-420 MA Body Composition Analyzer, Tanita Corporation, Tokyo, Japan.[10]

Analysis of the findings was carried out using the SPSS Program (Windows Release 18.0) (SPSS Inc., Chicago, Illinois, USA). Results were expressed as means $\pm$SEM, and comparison between groups was made using the two-tailed Student's t test. Linear and multiple regressions were used to evaluate the relative importance of the correlations among the various parameters.[11]

The study protocol had been approved by the Committee on Human Rights Related to Research Involving Human Subjects (Faculty of Medicine, Ramathibodi Hospital, Mahidol University). Written informed consent was obtained from all persons in the sample.

Results

Although the mean body mass indices were not significantly different between the male and female control subjects, the mean percentage of body fat of the women was significantly higher than that of the men, and the mean muscle mass, bone mass, and visceral fat of the male subjects were significantly higher than those of the females. The differences in body composition mentioned above were also found in the female controls and the male workers, but not when the male controls and male workers were compared (Table 1).

The findings of hemoglobin concentration, serum levels of triglyceride, and uric acid of the male workers and male controls were significantly higher than those of the female control subjects, whereas the HDL-cholesterol levels of female control subjects were significantly higher than those of both male groups. However, there were no significant differences in blood chemistry between male controls and male workers (Table 1).

Plasma adipokines (adiponectin, resistin, adipsin)

Table 2 displays the mean $\pm$SEM and range of plasma adiponectin, resistin, and adipsin levels. The mean $\pm$SEM of plasma adiponecin levels of the male controls was 17.6 $\pm$2.9 μg/mL, which was significantly higher than the 10.8 $\pm$1.5 μg/mL of the male workers ($p < 0.05$). The difference between the levels in females and male controls (11.0$\pm$2.1 μg/mL and 17.6$\pm$2.9 ug/mL, respectively) showed no significant difference ($p > 0.07$).

Table 1. Characteristics of subjects in the sample

	Mean ±SEM (range)		
Parameters	**Male tile workers**	**Females unexposed to asbestos**	**Males unexposed to asbestos**
Number	30	15	15
BMI (kg/m^2)	25.3±0.7 (20.3-33.3)	24.0 ±0.9 (19.0-31.1)	24.9 ±0.8 (19.3-31.6)
Total body fat (%bw)	21.7±1.0 (12.7-32.9)	32.1 ±1.6^{a1} (22.7-43.3)	20.9 ±1.1^{b1} (11.9-30.7)
Fat mass (kg)	15.3±1.1 (6.5-26.7)	18.9 ±1.6 (10.3-32.3)	15.0 ±1.3 (5.3-25.4)
Muscle mass (kg)	50.1±0.9 (40.7-60.1)	36.5 ±0.8^{a1} (31.9-42.3)	52.0 ±1.8^{b1} (37.2-62.3)
Bone mass (kg)	2.8±0.05 (2.3-3.3)	2.2 ±0.1^{a1} (1.8-2.8)	2.9 ±0.1^{b1} (2.1-3.4)
Visceral fat	11.8±0.8 (3-19)	5.7 ±0.62^{a1} (2-10)	9.7 ±1.0^{b2} (2-17)
Hemoglobin (g/dL)	14.1±0.2 (10.9-16.8)	12.1 ±0.3^{a1} (8.3-13.6)	14.5 ±0.3^{b1} (12.2-16.3)
Fasting blood sugar (mg/dL)	93.2+1.9 (71-109)	92±3 (77-108)	89 ±3 (74-117)
Serum			
LDL-cholesterol (mg/dL)	125±19 (58-179)	124 ±6 (63-184)	110 ±6 (74-142)
HDL-cholesterol (mg/dL)	50±1 (33-71)	57 ±3^{a2} (39-91)	48 ±3^{b4} (35-75)
Triglyceride (mg/dL)	142±19 (50-573)	103 ±10^{a1} (48-170)	196 ±32^{b3} (63-432)
Uric acid (mg/dL)	6.6±0.3 (4.0-11.1)	5.1 ±0.2^{a1} (3.8-6.5)	6.4 ±0.2^{b1} (5.0-8.3)

Significant difference compared with male tile workers : ^{a1} $p < 0.0005$, ^{a2} $p < 0.05$

Significant difference compared with female subjects unexposed to asbestos : ^{b1} $p < 0.0005$, ^{b2} $p < 0.005$, ^{b3} $p < 0.02$, ^{b4} $p < 0.05$

The mean ±SEM of plasma resistin levels of the female controls (0.101 ±0.012 μg/mL) was significantly higher than those of the male workers (0.065 ±0.008 μg/mL); that is, $p < 0.02$, while there was no significant difference between the female controls (0.101 ±0.012 μg/mL) and male controls (0.087 ±0.013 μg/mL); that is, $p > 0.45$.

The mean ±SEM of plasma adipsin levels of male workers (6.2 ±0.5 μg/mL) was significantly higher than those of the female controls (4.5 ±0.5 μg/mL); that is, $p < 0.05$, and was significant higher than in the male controls (4.2 ±0.3 μg/mL); that is $p < 0.0005$.

There were no significant differences in plasma adiponectin, resistin, and adipsin levels between the female and male controls, but when using the overall data of the two groups, i.e. workers and non-workers, the mean ±SEM plasma resistin level of the controls (0.094 ±0.009 μg/mL) was significantly higher than that of the male workers (0.065 ±0.008 μg/mL); that is, $p < 0.02$, whereas the plasma adipsin level of the controls (4.4 ±0.3 μg/mL) was significantly lower than that of the male workers (6.2±0.5 μg/mL); that is, $p < 0.005$. The plasma adiponectin levels were also not significantly different between the two groups (Table 3).

Table 4 displays the plasma adipsin levels (5.9 ±0.9 μg/mL) of the six workers in whom asbestos bodies had been detected,[12] compared with those in the 30 controls (4.4 ±0.3μg/mL), which was significantly higher at $p < 0.04$.

Discussion

The mean fat component of body composition in the female controls yielded total body fat (%bw) and fat mass (kg) levels significantly higher than those in the male controls and in the male workers. The opposite results were found for the fat-free component of body composition (muscle mass, bone mass) in the female controls, which was significantly lower than that in both male groups.

Routine blood chemistry examinations in the female controls were significantly different compared with both male groups. The results were interpreted as a natural phenomenon related to the sex of the subjects and their lifestyles.

Changes in plasma levels of adiponectin, resistin, and adipsin, while normally produced by adipose tissue, would also be secreted from macrophages and several other cells in response to an inflammatory process. No finding showed a relationship between the percentage of body fat, fat

Table 2. Plasma adiponectin, resistin, and adipsin levels of 30 male tile workers, and 15 female and 15 male subjects unexposed to asbestos

Parameters	Mean±SEM (range)		
	Male tile workers	Females unexposed to asbestos	Males unexposed to asbestos
Number	30	15	15
Plasma			
Adiponectin (μg/mL)	10.8±1.5 (2.3-35.6)	11.0 ±2.1 (3.0-33.0)	17.6 ±2.9[a3] (4.7-40.1)
Resistin (μg/mL)	0.065±0.008 (0.020-0.240)	0.101 ±0.012 [a2] (0.050-0.190)	0.087 ±0.013 (0.029-0.197)
Adipsin (μg/mL)	6.2±0.5 (2.5-17.0)	4.5 ±0.5 [a3] (2.6-10.6)	4.2 ±0.3[a1] (2.4-7.1)

Significant difference compared with male tile workers : [a1] $p < 0.0005$, [a2] $p < 0.02$, [a3] $p < 0.05$

Table 3. Plasma adiponectin, resistin, and adipsin levels of 30 tile male workers and 30 unexposed asbestos subjects

Parameters	Mean±SEM (range)	
	Male tile workers	Subjects unexposed to asbestos*
Number	30	30
Plasma		
Adiponectin (μg/mL)	10.8 ±1.5 (2.3-35.6)	14.3 ±1.8 (3.0-40.1)
Resistin (μg/mL)	0.065 ±0.008 (0.020-0.240)	0.094 ±0.009 [a2] (0.029-0.197)
Adipsin (μg/mL)	6.2 ±0.5 (2.5-17.0)	4.4 ±0.3 [a1] (2.4-10.6)

* 15 males and 15 females unexposed to asbestos.

Significant difference compared with male tile workers : [a1] $p < 0.005$, [a2] $p < 0.02$

mass, visceral fat, body mass index, and adipokines in this study. There were no significant differences in adiponectin, resistin, and adipsin levels between the male and female controls.

Plasma adiponectin in the male controls was significantly higher than that in the male workers and tended to be higher than in the female controls. This evidence may be due to the fact that adiponectin is a polypeptide that is highly specific to adipose tissue, in contrast to other adipocytokines, adiponectin levels are decreased in obesity. Therefore, adiponectin levels in this study are low in the obese, with body fat >25.0% body weight in males and >30.0% body weight in females;[13-16] we found the levels to be 13.3% (2/15) and 23.3% (7/30) of the male controls and the male workers, respectively.

The findings in other research studies[17-20] are in agreement with our findings that females unexposed to asbestos had a significantly higher percentage of body fat and resistin levels than that males unexposed to asbestos and male tile workers, except that the differences in resistin levels between females unexposed to asbestos tended to be higher than that in males, although they did not reach the level of significance. In addition, we found a significant negative correlation between plasma resistin levels and hemoglobin levels (r= -0.336, $p < 0.01$, n=60), thus this evidence confirmed that anemia is significantly more prevalent among obese individuals compared to non-obese ones.

This results of the study showed that, in the 30 male workers, plasma adipsin levels were significantly higher than those in female controls ($p < 0.05$) and in male controls ($p < 0.0005$). Because there was no significant difference by sex in the control groups, we combined them together. Leivo-Korpela et al.[7] studied 85 males with moderate to heavy occupational exposure to asbestos and unexposed controls. Their study showed that adipsin correlated positively with parenchymal fibrosis (r = 0.412, $p < 0.001$) and there was a linear increasing trend of mean plasma adipsin levels among the three groups of subjects exposed to asbestos (from normal parenchymal finding to borderline changes and fibrosis) ($p < 0.0001$).

Our results may suggest that adipsin would play a role as a biomarker for asbestos exposure. Comparisons were made between the 30 male workers and the 30 controls. No significant difference in adiponectin levels was found because the prevalence of obesity in these two groups was not significantly different (20.0% and 23.3%, respectively). We found that the mean resistin level in the control subjects was significantly higher than those in male workers ($p < 0.02$). This was due to the higher percentage of body fat in those subjects unexposed to asbestos than in the male tile workers (26.5 ±1.4%bw vs 21.7 ±1.0 %bw, $p < 0.01$). We observed a significant difference between the adipsin levels in the 30 control subjects and the 30 male workers ($p < 0.005$).

Table 4. Plasma adiponectin, resistin, and adipsin levels of 6 male tile workers positive for asbestos bodies and 30 subjects unexposed to asbestos

Parameters	Mean±SEM (range)		
	Male tile workers		Subjects unexposed to asbestos*
	with positive asbestos bodies	without asbestos bodies	
Number	6	24	30
Plasma			
Adiponectin	12.3 ±3.4	10.4 ±1.6	14.3 ±1.8
(μg/mL)	(4.6-28.1)	(2.3-35.6)	(3.0-40.1)
Resistin	0.066±0.020	0.069 ±0.009	0.094 ±0.009[b2]
(μg/mL)	(0.016-0.157)	(0.026-0.241)	(0.029-0.197)
Adipsin	5.9 ±0.9	6.2 ±0.6	4.4 ±0.3[a1,b1]
(μg/mL)	(3.4-8.4)	(2.5-17.0)	(2.4-10.6)

* 15 males and 15 females unexposed to asbestos

Significant difference compared with male tile workers positive for asbestos bodies : [a1] $p < 0.04$

Significant difference compared with male tile workers not positive for asbestos bodies : b1 $p < 0.02$, b2 $p < 0.03$

When comparisons of plasma adipsin levels were made between the 30 controls and the 6 male workers who had asbestos bodies, only a mild significant difference was found (p <0.04)**.** This finding suggests that plasma adipsin may be used as a biomarker in person exposed to asbestos, and that the plasma adipsin level is not dependent on the amount of body fat tissue. Nonetheless, a separate study is needed to verify whether the adipsin level is related to macrophages and other cells that secrete adipokines in response to inflammation in those cells. In this study, plasma adiponectin and resistin levels depended on the percentage of body fat. Also in this study, significant positive correlations between serum triglyceride and plasma adipsin levels were found, as well as serum uric acid and plasma adipsin levels.

The highest values of serum triglycerides (573 mg/dL), serum uric acid level (11.1 mg/dL), and plasma adipsin level (17.0 μg/dL) were found in a male worker who had been working in the factory for 35 years; he was obese (BMI=27.5 kg/m^2), had a relatively high percentage of body fat (23.4% bw), and high visceral fat level (14).

Conclusions

The results of the present study indicate that adiponectin levels are decreased in the obese. In addition, the obese subjects had higher resistin levels and lower hemoglobin levels than the non-obese. Although, the plasma adipsin levels in factory workers were apparently higher than those of the control subjects, suggesting that the determination of plasma adipsin might be useful as a biomarker for asbestos exposure, needs substantiation from further studies. The plasma adipsin levels did not correlate with the amount of body fat.

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