Differences in Levels of Prostate Specific Antigen and Insulin-like Growth Factor 1 in GSTP1 Gene Polymorphism among Workers

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Abstract

Cadmium (Cd) is classified as a carcinogen in humans (IA). In addition to causing lung cancer, the incidence of prostate cancer due to Cd exposure based on epidemiological research has also increased. This study aims to identify the GSTP1 genotypic frequency distribution and its correlation with PSA and IGF-1 levels in Cd exposed and unexposed workers. The research design used was cross sectional in 23 exposed groups (weld workers) and 40 unexposed groups (office employees) with a total of 63 people. The measurement of PSA and IGF-1 levels was carried out using the ELISA method and identification of the GSTP1 gene polymorphism using the PCR-RFLP method. Data analysis using Mann-Whitney and Spearman correlation test. The results showed that there was a relationship between PSA levels and IGF1 ($p < 0.01$, $r = 0.515$) in all subjects. The results of identification of GSTP1 gene polymorphism were obtained as ile/ile genotypes as much as 30.2% and ile/val as much as 69.8%. There were no differences in PSA and IGF1 levels between ile/ile and ile/val genotypes ($p > 0.05$). There was a relationship between PSA and IGF1 ($p < 0.01$, $r = 0.569$) in the group of Cd exposed workers and no differences in PSA and IGF1 levels between the ile/ile and ile/val genotypes ($p > 0.05$). There was a relationship between PSA and IGF1 ($p < 0.05$, $r = 0.342$) in the unexposed group of workers and no differences in PSA and IGF1 levels between ile/ile and ile/val genotype ($p > 0.05$).

Keywords: PSA, IGF-1, GSTP1 gene polymorphism, worker

Introduction

Cadmium (Cd) is thought to cause prostate cancer because in addition to the kidneys and liver, Cd is also accumulated in the prostate and testes. Several studies has found the association between Cd and prostate cancer deaths (Julin *et al*., 2012; Lin *et al*., 2013). Prostate Specific Antigen (PSA) can be used to detect early stage prostate cancer, clinical and monitoring after therapy with a limit of 4 ng / ml. Prostate specific antigen is a protein that is normally secreted exclusively by the prostate gland to help nourish sperm. However, if there is an increase in PSA levels above the normal level detected, it can be ascertained that there is a problem in the prostate. These problems can include malignancy (prostate cancer) or benign abnormalities (prostatitis and benign prostatic hyperplasia). After the use of PSA examination in the clinical world there was an increase in detection of prostate cancer by about 81% compared to before which only uses rectal examination (Baade *et al*., 2009).

Cd causes prostate cancer through several mechanisms, the initiation of malignant transformation, the nature of Cd as a substance that has activities such as androgen, antiapoptotic and mitogenic (Aimola *et al*., 2012; Arriazu *et al*., 2013; Lacorte *et al*., 2011). The antiapoptotic and mitogenic mechanism of Cd as cause of prostate cancer can be assessed with the Insulin Growth Factor1 (IGF1) indicator. Men with the highest quartile IGF1 levels have a 2.6 risk of prostate cancer (Chokkalingam *et al*., 2001).

Prostate cancer growth is affected by the presence of oxidative stress and reactive oxygen species. One of the genes involved in carcinogen detoxification and antioxidant activity is Glutathione S-transferase P1 (GSTP1) (Qadri *et al*., 2011). The presence of a polymorphism in this gene will cause a decrease in the elimination of carcinogens and may increase IGF1 and PSA levels. The aim of the study was to determine the differences PSA and IGF1 levels on the GSTP1 gene polymorphism in the Cd exposed group and unexposed group.

Method

This study was part of Cd carcinogenesis study on worker with cross sectional design. The research was carried out in 8 months (January to August 2015) and was located in Purwokerto and Banyumas City, Banyumas District, Central of Java, Indonesia. The subjects of the research came from two groups of workers. The Cd exposure group were 23 welding workers and the group of workers unexposed to Cd were 40 office workers. Samples were selected by consecutive sampling in accordance with the inclusion criteria, which were males over 30 years of age and having worked in the workplace for a minimum of 6 months. The exclusion criteria was acquiring an acute illness (elevated body temperature) during the last 4 months.

Blood samples were collected from the median cubital vein. Prior to blood sampling, the subjects were asked to fast for 10 hoursand not to have sexual intercourse during 2x24 hours. PSA and IGF-1 levels were determined using human Elisa kit (Sunred Biotechnology Company, Ltd, Shanghai), based on the principle of the double-antibody sandwich technique, and assayed on an Elisa Reader 270 (Biomeureux, France).

The exon 5 polymorphic site in GSTP1 locus (Ile105Val) was detected by restriction fragment length polymorphism (RFLP) of PCR-amplified fragments.The primers used were: P105F: 5'-ACC CCA GGG CTC TAT GGG AA-3' and P105R: 5'-TGA GGG CAC AAG AAG CCC CT-3'. PCR was carried out in a 30-μL vol-ume containing about 50 ng genomic DNA template, 200 μM of each dNTP, 200 ng of each primer, 1.5 mM MgCl2, 1X PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), and 1 U Taq DNA polymerase (Promega, Southampton, UK). After an initial denaturation step of 10 min at 95°C, the samples were processed through 30 temperature cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. A final extension step of 72°C for 10 min was performed. The 176-bp PCR products (20 μL) were digested for 2 h at 37°C with 2 U Alw26I (Fermentas Inc., Vilnius, Lithuania). The detection of the different alleles was carried out by horizontal 4% agarose gel electrophoresis with ethidium bromide, along with a 100-bp DNA ladder (Figure 1) (Qadri *et al*., 2011).

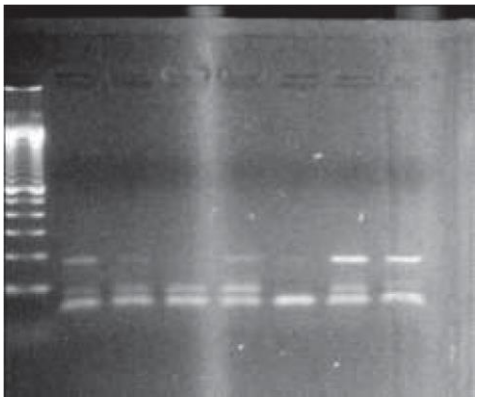

Figure 1. PCR-restriction fragment length polymorphism analysis of the GSTP1 Ile105Val polymorphism. The consensus sequence corresponding to the GSTP1 Iso allele was not cut, whereas the Val sequence corresponding to the GSTP1 Val allele was cleaved to yield two fragments (91 and 85 bp). Lane 6 = Wild-type homozygote (GSTP1 Ile/Ile); lanes 2-5 and lanes 7-8 = heterozygote (GSTP1Iso/Val); lane 1 = 100-bp DNA ladder.

Data analysis to determine differences between groups was by means of the MannWhitney test, because the data were not normally distributed. Data analysis to determine whether there was a correlation, was done using the Spearman correlation test.

Results

The mean age of exposed group was 40,31 ± 6,47, unexposed group was 38,50 ± 7,60 and the all subjects was 39,10 ± 7,01 years (Table 1).

Table 1. Subjects characterization

Group	Age (yr)
All workers (n=63)	39,10 ± 7,01
Exposed workers (n=23)	40,31 ± 6,47
Unexposed workers (n=40)	38,50 ± 7,60

The results of GSTP1 gene polymorphism were obtained as ile/ile genotypes 30.2% and ile/val 69.8% in all subjects, ile/ile genotypes 52.2% and ile/val 47.8% in exposed workers, ile/ile genotypes 17.5% and ile/val 82.5% in unexposed workers (Table 2).

Table 2. GSTP1 polymorphism distribution

GSTP1 polymorphism	Ile/ile	Ile/val
All workers (n=63)	19 (30,2)	44 (69,8)
Exposed workers (n=23)	12 (52,2)	11 (47,8)
Unexposed workers (n=40)	7 (17,5)	33 (72,5)

There were no differences in PSA and IGF1 levels between the ile/ile and ile/val genotypes (p> 0.05) in the all subjects, group of Cd exposed workers and unexposed workers (Table 3 and 4).

Table 3. Differences of PSA level

	Ile/ile	Ile/val	P
All workers (n=63)	3,05 ± 1,15	3,17 ± 4,11	0,095
Exposed workers (n=23)	3,63 ± 1,00	2,82 ± 1,37	0,406
Unexposed workers (n=40)	2,06 ± 0,58	2,64 ± 3,17	0,873

Table 4. Differences of IGF1 level

	Ile/ile	Ile/val	P
All workers (n=63)	6,54 ± 3,63	11,3 ± 25,09	0,952
Exposed workers (n=23)	7,75 ± 3,95	4,99 ± 2,29	0,065
Unexposed workers (n=40)	4,46 ± 1,74	9,46 ± 17,97	0,144

There was a relationship between PSA and IGF1 in the all subjects ($p < 0.01$, $r = 0.515$), Cd exposed workers ($p < 0.01$, $r = 0.569$) and unexposed workers ($p < 0.05$, $r = 0.342$) (table 5).

Table 5. IGF1 and PSA correlation

	p	r
All workers (n=63)	0,000*	0,515
Exposed workers (n=23)	0,005*	0,569
Unexposed workers (n=40)	0,031*	0,342

*significan p<0,05 (two-tailed)

Discussion

This study investigated the differences in levels of IGF1 and PSA levels on the GSTP1 gene polymorphisms between Cd exposed workers and unexposed workers. Since, the polymorphism in GSTP1 gene will decrease elimination of carcinogens, which can increased risk of prostate cancer. Insulin-like growth factors (IGF-I, IGF-II) and their binding proteins (IGFBP-1-6) play a key role in cell proliferation, differentiation and apoptosis, suggesting possible involvement in carcinogenesis. Meta-analysis confirms that raised circulating lGF-I is positively associated with prostate cancer risk (Rowlands *et al*., 2009).

We tried to confirm that glutathione S-transferase (GST) polymorphisms could enhanced oxidative stress on Cd exposed and unexposed workers. In our current study, we investigated 23 Cd exposed workers and 40 unexposed workers. We found the frequency of the ile/ile genotypes 30.2% and ile/val 69.8% in all subjects, ile/ile genotypes 52.2% and ile/val 47.8% in exposed workers, ile/ile genotypes 17.5% and ile/val 82.5% in unexposed workers. The range of Ile allele frequency was 0.47–0.86 in Africans, 63–76% in Europeans, 67–92% in Asians and 59–84% in Indians. The range of *Val105/Val105* allele was 14–53% in Africans, 23– 37% in Caucasians, 8–33% in Asians and 16–41% in Indians (Sharma *et al*., 2014). Qadri et al. found the frequency of the three different genotypes of *GSTP1 Ile105Val* in Kashmir ethnic population, i.e., Ile/Ile, Ile/Val and Val/Val, to be 52.4, 33.3 and 14.3% among prostate cancer cases, 48.5, 37.5 and 14% among benign prostate hyperplasia cases and 73.8, 21.3 and 5% in the control population. There was a significant association between the *GSTP1 Ile/Val* genotype and the advanced age group among the cases and conclude that *GSTP1 Ile/Val* polymorphism is involved in the risk of prostate cancer development (Qadri *et al*., 2011).

Circulating levels of IGF-I and its main binding protein, IGF binding protein 3 (IGFBP-3), have been associated with risk of several types of cancer. Whereas higher IGF-1 levels were associated with increased prostate cancer risk. The associations were primarily driven by lower-grade and non-advanced prostate cancer (Cao *et al*., 2015). There is no differences level of IGF1 between the ile/ile and ile/val genotypes in the all subjects, group of Cd exposed workers and unexposed workers.These findings were consistent with Henningson et al. in healthy women from breast cancer high-risk families (Lima *et al*., 2008).

In this study, there is no difference level of PSA between the ile/ile and ile/val genotypes in the all subjects, group of Cd exposed workers and unexposed workers. These were consistent with Lima et al. in the Brazilian population. There was no association between GSTP1 genotypes and possible clinical factors of risk or any parameter of tumour aggressiveness at diagnosis or during follow-up such as PSA (13). Meta-analysis by Wei et al. showed that GSTP1 Ile105Val polymorphism might not be significantly associated with overall prostate cancer risk (Wei *et al*., 2013).

IGF-1 modulates cell growth and survival, and is thought to be important in tumor development. The association between IGF-1 and prostate cancer risk is well established. Our study found there was correlation between IGF-1 and PSA. IGF-1 has been shown to stimulate the proliferation of human prostate epithelial cells in culture and to be necessary for normal growth and development of the rat and mouse prostate. Epidemiological studies have established a link between high circulating serum IGF-1 levels and the risk of later developing advanced prostate cancer, and overexpression of IGF-1 in the prostate basal epithelial layer of transgenic mice results in prostate adenocarcinoma that is similar to human disease. Thus, IGF-1 action appears to be important for prostate cancer initiation (Roberts , 2004).

Conclusion

Hence, in this study, we observed that IGF-1 and PSA in Cd exposed or unexposed workers was not affected by their glutathione S-transferase (GST) polymorphisms.

Acknowledgement

This work was supported by National Institute of Health Research and Development, Ministryof Health of the Republic of Indonesia.

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