Glutathione peroxidase in ovarian cancer patients in Indonesia

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Background. Glutathione peroxidase (GPx) is one of the antioxidant enzymes that maintain the balance of reactive oxygen species. GPx has a notable role in the progression of cancer, including ovarian cancer. Synthesis of this enzyme may be down-regulated in cases of ovarian cancer. As far as we are aware, this has not been studied in an Indonesian population.

Objective. To identify the difference in serum GPx levels between ovarian cancer patients and healthy controls.

Methods. This was an observational analytical study with a case–control design. The study was conducted in the Department of Obstetrics and Gynaecology at the Haji Adam Malik Hospital in Medan, Indonesia. Serum GPx levels were measured in 20 ovarian cancer patients and 20 control subjects.

Results. The types of ovarian cancer identified by histopathology in this study included serous adenocarcinoma (n=10; 50%) and various non-serous adenocarcinomas (50%). The mean (SD) serum GPx level was significantly lower in the cancer group (295.235 (244.479) mU/mL) than in the control group (743.546 (131.949) mU/mL) (p<0.0008). The median serum GPx level was lower among patients with serous ovarian cancer (209.915 mU/mL) than among those with non-serous ovarian cancer (338.885 mU/mL), although the difference was not statistically significant (p>0.226).

Conclusion. Serum GPx levels were found to be significantly lower in patients with ovarian cancer than in healthy controls. Further studies are needed to determine an appropriate cut-off level for serum GPx in ovarian cancer in this population.

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Ovarian cancer represents about 3% of cancers in women and is the fifth most common presentation of cancer in this demographic. A total of 22 280 new cases and 15 500 deaths occurred in the USA in 2012.[1] In Indonesia, cancer mortality was 136 per 100 000 in 2019.[2] More than 90% of ovarian cancers are epithelial. According to international data 30 - 70% of epithelial cancers are serous, 10 - 20% are endometrioid and 5 - 20% are mucinous. Clear-cell carcinomas account for 3 - 10% of ovarian cancers and 1% are undifferentiated.[3]

Molecular cancer studies have shown genetic alteration of oncogenes and tumour suppressor genes such as *BRCA1*, *p53*, *nm23* and *K-Ras* to be involved in the development of ovarian cancer. The alteration may be associated with the process of inflammation and oxidative stress.[4] Oxidative stress is caused by an imbalance between natural metabolic reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide radicals ($\cdot O_2^-$) and hydroxyl radicals ($\cdot$OH), and antioxidant enzymes and cofactors. ROS cause cellular damage to lipids, protein and DNA and are implicated in several stages of carcinogenesis (initiation, promotion and progression).[4,5] The increase of ROS in cancer cells stimulates cell proliferation, promotes gene mutation and affects cellular sensitivity to anticancer agents.[6] The increase also causes cells to lose their apoptotic ability, i.e. their ability to undergo natural cell death. Cellular defence mechanisms include free-radical scavengers, antioxidant enzymes and DNA repair enzymes.[7] Increases in ROS levels lead to the up-regulation of antioxidant enzymes, including glutathione peroxidase (GPx), glutathione-S-transferase, superoxide dismutase and catalase. These enzymes could serve as biomarkers of cancer progression.[6]

GPx is one of the antioxidant enzymes that react with H_2O_2.[8] Some studies have shown a decrease in GPx levels in patients with ovarian, breast, stomach, prostate or colorectal cancer.[9-11] Recent studies showed significant differences in GPx levels between patients with early-stage cancer and those in end stage/recurrence.[11] A previous study found that metabolism of glutathione xenobiotics (substances foreign to the organism, such as drugs) and antioxidants are deregulated early in the evolution of serous ovarian cancer.[12]

Our study aimed to compare GPx levels in ovarian cancer patients with those in healthy controls; to our knowledge, such a comparison has not been performed previously in an Indonesian population. This study also compared GPx levels in cases of serous and non-serous ovarian cancer.

Methods

This was an observational study with a case-control design, conducted in the Department of Obstetrics and Gynaecology at the Haji Adam Malik Hospital in Medan, Indonesia. The sample (N=40) included 20 patients with ovarian cancer and 20 healthy controls. Cancer patients were all new, sequential cases who presented to the department during the study period and who consented to inclusion in the study.

A blood sample (3 mL) was collected from each subject and centrifuged at 4 500 rpm for 10 minutes. Serum was then used for enzymatic estimation of GPx levels. Reaction mix (33 µL assay buffer, 3 µL 40 nM NADPH, 2 µL glutathione reductase and 2 µL glutathione) was added to each sample, the positive control (GPx) and the reagent control (assay buffer). To each 96-well plate coated

with serum, 40 µL assay buffer + 40 µL reaction mix was added for a final volume of 90 µL per well. Plates were incubated for 15 minutes, after which 10 µL of cumene hydroperoxidase was added to each well. Plates were assessed spectrophotometrically (340 nm) to obtain the first set of absorbance values, then incubated at 25°C for another 5 minutes and again read at 340 nm to obtain the second set of absorbance values. GPx levels of the patient group and the control group were compared statistically using an independent *t*-test. GPx levels of the serous cancer group and the non-serous group were compared using a Mann–Whitney *U*-test.

Ethical approval

The study was approved by the Ethics Committee Board of Haji Adam Malik Hospital (ref. no: 80/TGL/KEPK FK USU-RSUPHAM/2014).

Results

Demographic characteristics are shown in Table 1. Women between 40 and 50 years of age represented the largest subgroup in both the case group (*n*=20; 40%) and the control group (*n*=9; 45%). More subjects were premenopausal than postmenopausal (80.0% v. 17.5%), although the numbers were not statistically different ($p>0.05$). The majority of participants, in both the case group and the control group, were older than 13 years at menarche. There was no statistically significant association between age of menarche and the incidence of ovarian cancer ($p>0.05$). The majority of participants, in both groups, had had children.

Histopathology showed the types of ovarian cancer among patients to include serous adenocarcinoma (*n*=10; 50%), mucinous adenocarcinoma (*n*=8; 40%), dysgerminoma (*n*=1; 5%) and yolk sac tumour (*n*=1; 5%) (Table 2).

The mean GPx serum level was lower in the case group (295.235 mU/mL, 95% confidence interval (CI) 233.481 - 356.989 mU/mL) than in the control group (743.546 mU/mL, 95% CI 629.126 - 857.966 mU/mL) (Table 3). The difference was statistically significant ($p=0.008$).

The median GPx serum level was lower among patients with serous ovarian cancer (209.915 mU/mL, 95% CI 158.330 - 624.630 mU/mL) than among those with non-serous cancer (338.885 mU/mL, 95% CI 233.481 - 356.989 mU/mL). However, the difference was not statistically significant ($p>0.226$) (Table 4).

The GPx levels were raised in both cases of germ cell tumours: 436.11 mU/mL in the patient who presented with a yolk sac tumour and 446.03 mU/mL in the patient with dysgerminoma.

Table 1. Demographic characteristics of the subjects

Characteristics	Control group (*N*=20), *n* (%)	Case group (*N*=20), *n* (%)	Total (*N*=40), *n* (%)
Age (years)			
20 - 39	6 (30.0)	7 (35.0)	13 (32.5)
40 - 50	9 (45.0)	8 (40.0)	17 (42.5)
>50	5 (25.0)	5 (25.0)	10 (25.0)
Menopause status			
Menopausal	3 (15.0)	4 (20.0)	7 (17.5)
Premenopausal	17 (85.0)	16 (80.0)	33 (82.5)
Age at menarche (years)			
<13	5 (25.0)	3 (15.0)	8 (20.0)
≥13	15 (75.0)	17 (85.0)	32 (80.0)
Parity			
Virgo	3 (15.0)	3 (15.0)	6 (15.0)
0	1 (5.0)	2 (10.0)	4 (7.5)
≥1	16 (80.0)	15 (75.0)	30 (77.5)

Discussion

Oestrogen exposure and ovulation in premenopausal women and an early age of menarche (<12 years) are considered risk factors for ovarian cancer, as repetitive damage to and subsequent repair of the epithelial ovarian surface over a long period increase the risk of spontaneous mutation.[13] Other risk factors include inflammation, high levels of steroid hormones, hereditary factors, infertility, use of oral contraceptives (decreased risk), age, asbestosis, talc and reproductive factors such as nulliparity. We did not find any relationship between the incidence of ovarian cancer, menopause status and the age of menarche among participants in our study.

The most common histopathologically identified type of ovarian cancer is serous carcinoma, followed by endometrioid tumours, clear-cell carcinoma and mucinous tumours.[14] Serous adenocarcinoma was the most common histopathologically identified ovarian cancer in our study (50%).

This study showed that the level of GPx was lower in patients with serous adenocarcinoma than in those with non-serous cancer types, although the result was not statistically significant. A previous study showed that the synthesis of antioxidants is deregulated in serous ovarian cancer, probably owing to early genomic alterations mediated by *p53* and other oncogenes.[12]

We found that serum GPx levels were lower in the cancer patients than in the healthy controls. GPx acts as a protective agent against oxidative stress that could lead to DNA damage and therefore, possibly, the progression of cancer. ROS, including H_2O_2, $^{\bullet}O_2^-$ and $^{\bullet}OH$, are produced owing to follicular rupture during ovulation and can damage the DNA of the epithelial ovarian surface.[15] ROS also cause an increase in cellular proliferation and an increase in genetic mutation, which can result in genetic instability, increased cellular invasion and angiogenesis. These processes damage cellular components such as lipids, proteins and DNA, which have a role in carcinogenesis.[4]

Table 2. Types of ovarian cancer identified by histopathology (*N*=20)

Type of ovarian cancer	*n* (%)
Serous adenocarcinoma	10 (50.0)
Mucinous adenocarcinoma	8 (40.0)
Dysgerminoma	1 (5.0)
Yolk sac tumour	1 (5.0)

Table 3. Glutathione peroxidase serum levels in the case group and control group

Statistical parameter	GPx level (mU/mL)		*p*-value*
	Control group	Case group	
Mean (SD)	743.546 (244.479)	295.235 (131.540)	0.008
Minimum	629.126	233.481	
Maximum	857.966	356.989	

GPx = glutathione peroxidase; SD = standard deviation.
*Independent *t*-test.

Table 4. Glutathione peroxidase serum levels in serous cancer cases compared with non-serous cancer cases

	GPx level (mU/mL)		
Statistical parameter	**Serous ovarian cancer**	**Non-serous ovarian cancer**	***p*-value***
Median	209.915	338.885	0.226
Minimum	158.330	108.730	
Maximum	624.630	446.030	

GPx = glutathione peroxidase; SD = standard deviation.
*Mann–Whitney *U*-test.

A previous study found that expression of the gene encoding GPx is strongly down-regulated in endometrial tumour cell lines.[10] The down-regulation might be due to structural aberrations at the gene's locus, such as a deletion in the region of *RNO10* found by Nordlander *et al.*,[16] resulting in low expression of the gene. A recent study found a decrease of serum protein GPx3, a selenocysteine-containing antioxidant enzyme, in women with serous ovarian cancer.[11] In contrast, some studies have shown a higher expression of the gene coding for GPx in clear-cell ovarian cancer compared to controls and in other epithelial ovarian cancer cells.[17,18] This suggests an anomaly and demonstrates the need for further investigation in larger studies.

Conclusion

The current study shows that GPx is a molecular marker that is significantly decreased in ovarian cancer in an Indonesian sample, especially in serous adenocarcinoma. Further studies are needed to determine a cut-off level of serum GPx levels in ovarian cancer and to clarify the possible differences between various forms of this cancer. GPx levels may provide therapeutic targets and possibly aid in the diagnosis of ovarian cancer. Our results regarding GPx levels in different tumour types should be interpreted with caution owing to the relatively higher incidence of mucinous tumours seen compared with what is reported in published data.

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1. American Cancer Society. Cancer Facts & Figures 2012. Atlanta: ACS, 2012. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2012.html (accessed 21 August 2018).
2. Ministry of Health of the Republic of Indonesia. World Cancer Day. http://www.depkes.go.id/article/view/19020100003/hari-kanker-sedunia-2019.html (accessed 20 October 2019).
3. Sorbe B, Frankendal B, Veress B. Importance of histologic grading in the prognosis of epithelial ovarian carcinoma. Obs Gynecol 1982;59(5):576-582.
4. Senthil K, Aranganathan S, Nalini N. Evidence of oxidative stress in the circulation of ovarian cancer patients. Clin Chim Acta 2004;339(1-2):27-32. https://doi.org/10.1016/j.cccn.2003.08.017
5. Rashid K, Sinha K, Sil P. An update on oxidative stress-mediated organ pathophysiology. Food Chem Toxicol 2013;62:584-600. https://doi.org/10.1016/j.fct.2013.09.026
6. Pelicano P. ROS stress in cancer cells and therapeutic implications. Drug Resist Updat 2004;7(2):97-110. https://doi.org/10.1016/j.drup.2004.01.004
7. Kryston TB, Georgiev AB, Pissis P, Georgakilas AG. Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res 2011;711(1-2):193-201. https://doi.org/10.1016/j.mrfmmm.2010.12.016
8. Abdalla M. Glutathione as potential target for cancer therapy: More or less is good? Jord J Biol Sci 2011;4:119-124. jjbs.hu.edu.jo/files/v4n3/Paper_number1_modified-Final.pdf
9. Abdel-Aziz AF, El-Refaeey AA, Negm A, El-Taweel HMA. Role of human beta defensin-2 and oxidative stress parameters in ovarian cancer patients. Int J Biochem Res Rev 2013;3(4):364-379. https://doi.org/10.9734/ijbcrr/2013/5105
10. Falck E, Karlsson S, Carlsson J, Helenius G, Karlsson M, Klinga-Levan K. Loss of glutathione peroxidase 3 expression is correlated with epigenetic mechanisms in endometrial adenocarcinoma. Cancer Cell Int 2010;10:46-54. https://doi.org/10.1186/1475-2867-10-46
11. Agnani D, Vanegas-Camacho O, Camacho C, et al. Decreased levels of serum glutathione peroxidase 3 are associated with papillary serous ovarian cancer and disease progression. J Ovarian Res 2011;4:18. https://doi.org/10.1186/1757-2215-4-18
12. George S, Nelson O, Sowamber R, et al. Antioxidant gene expression program is deregulated early in serous ovarian cancer. Gynecol Oncol 2017;145(Suppl 1):130. https://doi.org/10.1016/j.ygyno.2017.03.304
13. Berek J, Hecker N, Friedlander M. Epithelial Ovarian, Fallopian Tube, and Peritoneal Cancer. In: Berek JS, Hacker NF, eds. Berek and Hacker's Gynecologic Oncology. 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2010:508-543.
14. Rosen D, Yang G, Liu G, et al. Ovarian cancer: Pathology, biology, and disease models. Front Biosci 2010;14:2089-2102. https://doi.org/10.2741/3364
15. Purdie DM, Bain CJ, Siskind V, Webb PM, Green AC. Ovulation and risk of epithelial ovarian cancer. Int J Cancer 2003;104(2):228-232. https://doi.org/10.1002/ijc.10927
16. Nordlander C, Karlsson S, Karlsson Å, et al. Analysis of chromosome 10 aberrations in rat endometrial cancer – evidence for a tumor suppressor locus distal to Tp53. Int J Cancer 2007;120(7):1472-1481. https://doi.org/10.1002/ijc.22533
17. Lee HJ, Do JH, Bae S, et al. Immunohistochemical evidence for the over-expression of glutathione peroxidase 3 in clear cell type ovarian adenocarcinoma. Med Oncol 2011;28(Suppl 1):522-527. https://doi.org/10.1007/s12032-010-9659-0
18. Hough CD, Cho KR, Zonderman AB, Schwartz DR, Morin PJ. Coordinately up-regulated genes in ovarian cancer. Cancer Res 2001;61:3869-3876.

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