The Appraisalment of Antioxidant and Oxidant Status in Women Undergoing Surgical Menopause

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Abstract The present study was undertaken to study the impact of surgical menopause on oxidant and antioxidant status in relation to estrogen levels after 3 months of surgery. Total 130 women who had undergone total hysterectomy (TH) with or without bilateral salpingo-oophorectomy (BSO) were included in this study. The oxidant status was assessed by measuring plasma levels of malondialdehyde and antioxidant status was assessed by measuring superoxide dismutase, catalase, glutathione, glutathione peroxidase, estrogen, and Vitamin A, E and C levels. The malondialdehyde level was significantly increased ($p < 0.05$) in all women who underwent TH with or without BSO. Significant increased levels of superoxide dismutase were observed in women who underwent TH with BSO. The blood glutathione levels were significantly decreased in women after TH only but significantly increased in women who had undergone TH with BSO. The levels of estrogen, vitamin E and vitamin C were significantly decreased in

women who underwent TH with BSO. The catalase, GPx and vitamin A did not differ significantly in all groups. The result suggests that surgical menopause is associated with oxidative stress which reiterates the fact that ovaries retain some function even after menopause.

Keywords Surgical menopause · Malondialdehyde · Superoxide dismutase · Catalase · Glutathione · Estrogen

Introduction

Menopause is a permanent ovarian senescence that occurs during fourth to fifth decade of life and is associated with decreased levels of estrogen. An increase in serum follicle-stimulating hormone (FSH) and decrease in estradiol and inhibin are the major endocrine changes that occur during the transition to menopause [1, 2]. Surgical removal of the ovaries also stops estrogen production suddenly, leading to more severe symptoms than those generated by natural menopause like hot flashes, night sweats, breast tenderness, vaginal dryness, irregular menses, mood changes, vaginal atrophy, osteoporosis, heart disease and sometimes premature ovarian failure [3–5].

Total Hysterectomy (TH) is the surgical removal of uterus along with cervix for various indications like dysfunctional uterine bleeding, menstruation irregularities, uterine prolapse, and fibroids. Bilateral salpingo-oophorectomy (BSO) is usually done along with TH to prevent the future development of ovarian cancer [6]. The TH rate is higher in women who are less than 55 years of age, with BSO in 37 % of women who are less than 45 years of age and 68 % in women aged 45 years or older [7].

The BSO causes a rapid decline in circulating levels of ovarian estrogen and androgens in premenopausal women.

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Even in postmenopausal women, BSO results in significant lower levels of testosterone and androstenedione which are converted to estrogen peripherally. Estrogen depletion caused by BSO is associated with increased reactive oxygen species (ROS) leading to various disorders such as coronary heart disease, hip fractures as well as higher risk of cognitive impairment, depression and anxiety [8–10].

Estrogens are known to function as an antioxidant and free radical scavenger [11]. The estrogen depletion due to BSO causes down regulation of antioxidant gene expression which in turn affects circulating redox state and results in oxidative stress [12]. However, this stress is countered by estrogen as observed during ovaries retention [13, 14] or by estrogen replacement therapy which restores total plasma antioxidant capacity and decreases lipid peroxidation [12, 15, 16]. The estrogen influences the circulating redox balance via regulating the activity of the cellular antioxidative enzyme system such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) which transforms free radicals into less reactive or inactive species. Various studies have reported that endogenous estrogen from retained ovaries reduces the risk of ovarian cancer [17], coronary heart disease [18], osteoporotic hip fracture [19] and breast cancer [20], even in the postmenopausal group. The loss of endogenous estrogen also increases the level of LDL-C (Low density lipoprotein cholesterol) due to the increase in PCSK9 (Proprotein convertase subtilisin/kexin type 9) levels thus affecting lipid and lipoprotein metabolism [21].

Various studies on animals have reported an increased risk of developing oxidative stress after oophorectomy [22, 23]. However, there is very scanty literature available for establishing the relationship between surgical menopause, oxidative stress and antioxidant status in women. Kaur et al. (2006) [13] did preliminary study on 38 women who underwent TH with or without BSO and reported that there is oxidative stress of surgery in both premenopausal and postmenopausal women. They also found that in case of retained ovaries (only TH), the ovaries responded to the surgery by increasing estrogen production after 45 days of surgery. However, there is a need for long terms follow up studies with large sample size. In view of these facts, we aimed to study and compare the oxidative stress and role of antioxidant (enzymatic and non-enzymatic) in post and premenopausal women undergoing TH (with or without BSO) and estrogen levels, which will help to evaluate the impact of surgical menopause on oxidant and antioxidant status after 3 months of surgery.

Methods

The aim of the study was to observe the surgical impact of TH (removal of uterus) with or without BSO (removal of ovaries) on oxidant and antioxidant status in relation to

estrogen levels after 3 months of surgery. Total one hundred and thirty (130) women were included in this study that had undergone TH with or without BSO at the Obstetrics and Gynaecology Department, GMCH, Sector-32, Chandigarh, India. Informed consent was obtained before recruitment according to the guidelines of the institutional ethics committee. They were categorized into four groups as follows; I) premenopausal women undergoing TH, II) premenopausal women undergoing TH with BSO, III) postmenopausal women undergoing TH and IV) postmenopausal women undergoing TH with BSO. The mean age of premenopausal women undergoing TH (N = 50) was 41.23 ± 4.079 years and those undergoing TH with BSO (N = 30) was 44.36 ± 4.319 years. In postmenopausal group, the mean age of women undergoing TH (N = 30) was 58.89 ± 9.816 years whereas the mean age of women undergoing TH with BSO (N = 20) was 56.8 ± 4.438 years. The average age of attaining menopause in premenopausal group was around 15.89 ± 9.6 .

Women with conditions such as endometriosis or ovarian cysts, or malignancies of the reproductive organs were excluded. The women included in our study have no history of smoking or alcohol consumption. Women who required hormone therapy following surgery for acute menopausal symptoms were also excluded. About 5 ml of heparinized venous blood was collected from the subject before surgery and after 3 months of surgery. The erythrocytes were washed with cold isotonic saline and used for estimation of SOD, CAT, GPx and plasma was used for estimation of MDA, Vitamin A, E and C levels. Heparinized blood was used for estimation of GSH levels.

The oxidative stress was determined by the assessment of the plasma MDA level according to the method of Ohkawa et al. [24]. The value of MDA was expressed as $\mu\text{mole/mg}$ protein. SOD activity in plasma was measured by modified method of Nishikimi et al. [25]. Activity of SOD was expressed in units of enzyme/mg protein, where one unit was defined as the amount of SOD required to inhibit the rate of reduction of nitrobluetetrazolium by 50 %. The CAT activity in erythrocytes was measured by the method of Prasad et al. [26]. CAT catalyses the breakdown of hydrogen peroxide (H_2O_2) which was measured at 240 nm and was expressed in $\mu\text{moles/min/g Hb/L}$. GSH in blood was determined according to the procedure described by Beutler et al. [27]. The protein free filtrate obtained after precipitating with metaphosphoric acid produces a relatively stable yellow-coloured solution on reaction with DTNB (5, 5'-dithiobis-nitro benzoic acid), which was measured at 412 nm and the value was expressed in mg/g Hb . Vitamin C level in plasma was measured by the method described by Frei et al. [28], which was recorded at 520 nm and the concentration of vitamin C was expressed in $\text{mg \%}$. The plasma levels of vitamin A and E were measured using high pressure

liquid chromatography (HPLC) according to the previously described method (Kaur et al., 2008) using Waters 1525 Binary HPLC Pump and Waters 2487 Dual λ Wavelength Detector. α -tocopherol and retinol acetate manufactured by Sigma Alderich Co. were used as standards respectively for vitamin E and vitamin A. The values were expressed as $\mu\text{g/ml}$. The serum estrogen level in each subject was estimated as per the manufacturer instruction by the enzyme linked immunoassay (ELISA) using ADALTIS EIA GEN Estradiol Kit [ADALTIS ITALIA].

The data obtained for each parameter were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using SPSS software, version 18. The data obtained for each group were subjected to one way ANOVA to test the significance of variance among the groups and the unpaired Student's *t* test was applied using SPSS software to test the significance of difference.

Results

In the present study, the oxidative stress was assessed by determination of MDA level after 3 months of surgery. The MDA levels were significantly increased ($p < 0.05$) in

Fig. 1 The graph shows the level of MDA before (black) and 3 months after surgery (white) of TH and TH with BSO in pre and postmenopausal women (* $p < 0.05$)

Fig. 2 The graph shows the level of SOD before (black) and 3 months after surgery (white) of TH and TH with BSO in pre and postmenopausal women (* $p < 0.05$)

both premenopausal and postmenopausal women who underwent TH with or without BSO (Fig. 1). There was significant increase in activity of SOD ($p < 0.05$) in both premenopausal and postmenopausal women who underwent BSO during TH (Fig. 2) but there was no change in the SOD activity in both premenopausal and postmenopausal women who underwent TH only. There was a decrease in the catalase levels in both premenopausal and postmenopausal women who underwent TH with or without BSO. However, the catalase levels did not differ significantly before and after surgery in both premenopausal and postmenopausal women in all the groups (Fig. 3). The Glutathione peroxidase levels decreased in both premenopausal and postmenopausal women who underwent surgery i.e. TH and TH with BSO. However, the levels did not differ significantly in all the groups. The blood GSH levels were significantly decreased in both premenopausal and postmenopausal women after TH alone. However, the blood GSH levels were significantly elevated in premenopausal and postmenopausal women after TH with BSO (Fig. 4). The plasma estrogen levels were estimated to find a correlation between oxidant, antioxidant and estrogen levels in all the groups. The level of estrogen was decreased significantly in patients who underwent BSO during TH ($p < 0.05$), whereas there was no significant change in estrogen levels in response to TH in both premenopausal and postmenopausal women (Fig. 5). Since vitamins such as A, E and C affect oxidative status by preventing glycosylation and peroxidation of protein synthesis of antioxidant enzymes [29], an attempt has been made to estimate the levels of vitamins A, E and C in all the groups. There was a decrease in the levels of vitamin A but not significant in all the groups. The level of vitamin E and C was significantly decreased in both pre and postmenopausal patients where oophorectomy was done during TH (Figs. 6, 7). However, there was no significant decrease in the levels of vitamin E and C in patients (both pre and postmenopausal) where only hysterectomy was done.

Fig. 3 The graph shows the level of catalase before (black) and 3 months after surgery (white) of TH and TH with BSO in pre and postmenopausal women (* $p < 0.05$)

Discussion

Surgical menopause is associated with a significant change in the estrogen levels which in turns affects circulating redox state [12, 13]. This leads to imbalance between

oxidants (superoxide anion radical, hydrogen peroxide, hydroxyl radical, peroxynitrite) and antioxidant defence system (enzymatic and non-enzymatic). The enzymes SOD, CAT, and GSH-Px work synergistically to maintain an optimal redox balance by transforming reactive free radicals into less reactive or inactive species [30]. Estrogen is the most potent endogenous antioxidant and estrogen levels regulate the activity of cellular antioxidative enzyme system by inducing antioxidant enzyme expression [12].

Various studies have reported that the surgical menopause is associated with the increased risk of development of the oxidative stress which could lead to various diseases such as heart disease, osteoporosis, Alzheimer's disease, coronary artery disease and depression. Estrogen replacement is able to prevent and counteract such oxidative stress [12]. Estrogens have been shown to have in vitro antioxidant effects on membrane phospholipid peroxidation [31] as supplementation of estrogen after menopause decreases serum lipid peroxides and restores total plasma antioxidant capacity [18, 24].

Fig. 4 The graph shows the level of reduced glutathione GSH before (black) and 3 months after surgery (white) of TH and TH with BSO in pre and postmenopausal women (* $p < 0.05$)

Fig. 5 The graph shows the level of estrogen before (black) and 3 months after surgery (white) of TH and TH with BSO in pre and postmenopausal women (* $p < 0.05$)

Fig. 6 The graph shows the level of Vit E before (black) and 3 months after surgery (white) of TH and TH with BSO in pre and postmenopausal women (* $p < 0.05$)

Fig. 7 The graph shows the level of Vitamin C before (black) and 3 months after surgery (white) of TH and TH with BSO in pre and postmenopausal women (* $p < 0.05$)

In the present study increased oxidative stress was observed after 3 months of surgery in both pre and postmenopausal women undergoing BSO. The increased levels of MDA were reported earlier in pre and postmenopausal women undergoing TH as well as BSO after 30–45 days of surgery [12, 13]. The oxidative stress could be due to increase in H_2O_2 which might have induced the peroxidation of polyunsaturated fatty acids and lead to formation of MDA, a by-product of peroxidation. The peroxidation of lipids can dramatically change the properties of biological membranes [32, 33]. In ovariectomized rats, increased oxidative stress has been reported in terms of augmented levels of MDA [34, 35]. Stupka and Tiidus [36] reported that there was a relationship between decreased estrogen and MDA in ovariectomized rats and ovariectomy led to increased free radical production. Various studies have reported that estrogen therapy along with vitamin E and C treatments is effective in ameliorating the levels of MDA in various tissues in ovariectomized rats [29] and in premenopausal women [37] as well as in postmenopausal women [17].

There were increased SOD levels in both pre and postmenopausal women after 3 months of surgery but the levels were more significant in patients who had undergone TH with BSO. However, there was no significant decrease in GPx activity in any of the groups. Arslan et al. [34] also reported increased plasma SOD levels after 100 days of ovariectomy in rats. The increased activity of SOD could lead due to conversion of superoxide into H_2O_2 which is decomposed by CAT into H_2O and O_2 . Increase in SOD activity with unchanged GPx activity in ovariectomized rats could result in ROS formation resulting in oxidative stress which plays an important role in pathogenesis of various diseases such as coronary heart disease, hip fractures and other cognitive impairment. Glutathione and other thiols maintain the redox balance of cells, thereby preventing oxidative damage. Various other studies on ovariectomized rats also reported no significant change in plasma GPx levels [31, 34].

There was a decrease in the level of catalase in all groups which could be explained on the basis of the fact that this enzyme has been utilized against increased production of H_2O_2 from superoxide radicals by SOD enzyme. Catalase plays an important role in adaptive response to oxidative stress [38] and also levels of catalase are influenced by superoxide anion [39]. However, the GSH levels were significantly increased in patients with TH with BSO in both premenopausal and postmenopausal women which could be due to compensatory response to elevated oxidative stress induced by the surgery [13]. The enhanced production of reduced glutathione which is an intracellular antioxidant system plays a key role in cellular detoxification reactions and in regulating the thiol-disulfide cellular status [12].

The reduced levels of Vitamin E and C levels were observed in all groups and more significantly in patients with TH with BSO in both premenopausal and postmenopausal women who signifies enhanced oxidative stress

in these groups. Vitamin E, A, C plays a major role in lipid membrane protection and also possesses ROS-scavenging chain-breaking antioxidant activity like estrogen [30]. The decrease in levels of Vitamin C might be due to its increased consumption to counteract oxidative stress and inhibit lipid peroxidation. Under invitro conditions, estradiol regenerates oxidized tocopherol radicals by donating a hydrogen atom of the OH group at aromatic ring [36].

The estrogen levels were found to be decreased as reported by Kaur et al. [13] in both postmenopausal and premenopausal women following BSO. Bellatini et al. [12] also reported decreased estrogen levels in premenopausal women undergoing BSO and increased oxidative stress which can be prevented by estrogen replacement therapy. However, there was no change in estrogen levels in both post and premenopausal women where ovaries were retained. Kaur et al. [13] reported increased estrogen levels after 45 days of surgery. However, in the present study the estrogen levels returned to the baseline levels after 3 months of surgery. These substantiates the findings of earlier studies by Kaur et al. [13], that endogenous estrogen is the first line of defence against oxidative stress and could be better antioxidant than GSH.

Conclusion

The present study observed that the endogenous estrogen from the retained ovaries plays a critical role in combating the oxidative stress by regulating the intricate defensive mechanism of natural antioxidant system of the body such as GSH, SOD enzymes and vitamin E and C. It also reiterates the fact that ovaries retain some functions even after menopause and attempt should be made to retain ovaries in women younger than 65 years with low risk of malignancies in breast and ovary as recommended by American College of Obstetricians and Gynaecologists [40].

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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