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# Aberrations in one-carbon metabolism induce oxidative DNA damage in sporadic breast cancer

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**Abstract** The authors investigated the role of dietary micronutrients and eight functional polymorphisms of one-carbon metabolism in modulating oxidative stress in sporadic breast cancer. PCR-restriction fragment length polymorphism (RFLP) and PCR-amplified fragment length polymorphism (AFLP) methods were used for genetic analysis in 222 sporadic breast cancer cases and 235 controls. Standardized food frequency questionnaire was used for dietary micronutrient assessment. 8-oxo-2′-deoxyguanosine (8-oxodG), folate, and estradiol were estimated using commercial ELISA kits. Reverse-phase HPLC coupled with fluorescence detector was used for plasma homocysteine analysis. Total glutathione was estimated using Ellman's method. Reduced folate carrier 1 (RFC1) G80A and methylenetetrahydrofolate reductase (MTHFR) C677T were associated with risks of 1.34 (95% CI 1.01–1.79)- and 1.84 (95% CI 1.14–3.00)-folds, respectively, for sporadic breast cancer while cytosolic serine hydroxymethyl transferase (cSHMT) C1420T was associated with reduced risk (OR 0.71, 95% CI 0.53–0.94). Significant increase in plasma 8-oxo-2′-deoxyguanosine ($P < 0.004$) and homocysteine ($P < 0.0001$); and significant decrease in total glutathione ($P < 0.01$) and dietary folate ($P = 0.006$) was observed in cases than in controls. Oxidative DNA damage showed direct association with menopause ($P = 0.02$), RFC1 G80A ($P < 0.05$) and homocysteine ($P < 0.0001$); and inverse association with dietary folate ($P < 0.0001$), plasma folate ($P < 0.0001$), cSHMT C1420T ($P < 0.05$) and glutathione ($P < 0.001$). To conclude, the aberrations in one-carbon metabolism induce oxidative stress in sporadic breast cancer either by affecting the folate pool or by impairing remethylation.

**Keywords** Sporadic breast cancer · 8-oxo-2′-deoxyguanosine · One-carbon metabolism · Polymorphisms · Dietary micronutrients

**Abbreviations**

| | |
|---|---|
| 8-oxodG | 8-Oxo-2′-deoxyguanosine |
| AFLP | Amplified fragment length polymorphism |
| E2 | Estradiol |
| GCPII | Glutamate carboxypeptidase II |
| MTR | Methionine synthase |
| MTRR | Methionine synthase reductase |
| MTHF | Methylene tetrahydrofolate |
| MTHFR | Methylenetetrahydrofolate reductase |
| PCR | Polymerase chain reaction |
| RFC1 | Reduced folate carrier 1 |
| RFLP | Restriction fragment length polymorphism |
| cSHMT | Cytosolic serine hydroxymethyltransferase |
| tHcy | Total plasma Homocysteine |
| THF | Tetrahydrofolate |
| TYMS | Thymidylate synthase |

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## Introduction

Evidence from the several epidemiological studies indicated increased oxidative stress and the aberrations in one-carbon metabolism as etiological factors for the breast cancer [1, 2]. Studies indicated that diet rich in fruits and vegetables decreases the oxidative stress and may help us to prevent the development of breast carcinoma because of the presence of anti-oxidants [3]. Oxidative stress may result in oxidative DNA damage, lipid peroxidation, protein modification, membrane disruption, and mitochondrial damage [4]. Higher levels of 8-oxo-2′-deoxyguanosine (8-oxodG) were reported in the breast tissue and lymphocyte DNA of the breast cancer cases than of control subjects [5]. Increased excretion of 8-oxodG was found to lower breast cancer risk by ~30% [6].

One-carbon metabolism is a network of interconnected biological reactions in which "one-carbon moiety (formyl/methylene/methyl)" is transferred from one substrate to other to form crucial metabolites that react either with the pro-oxidants or promote anti-oxidant defense. Cofactors of one-carbon metabolism, specifically folate and B12 were reported to be effective in reducing the arsenic-induced oxidative damage by regaining the activities of anti-oxidant defense enzymes, such as the superoxide dismutase (SOD) and catalase, and by increasing the levels of anti-oxidant glutathione [7]. Further, long-term depletion of folate/methyl from the diet was shown to decrease reduced/oxidized glutathione ratio, alter activities of Mn-SOD, catalase, and glutathione peroxidase, and induce irreparable oxidative DNA damage [8]. Further, hyperhomocysteinemia, a consequence of aberrations in one-carbon metabolism, was found to increase the superoxide production by multiple mechanisms [9]. *S*-adenosylmethionine (SAM), the end product of one-carbon metabolism, was reported to increase the activities of SOD and glutathione-S-transferase (GST), and restore glutathione levels [10]. In hereditary breast cancer cases, BRCA1 mutations render the protein incapable of repairing DNA double-strand breaks and inhibiting BRCA1-mediated upregulation of anti-oxidant defense enzymes, which might be responsible for the increased oxidative stress in BRCA1 mutants [11, 12]. On the contrary, sporadic breast cancer cases have active BRCA1 and any alteration in the oxidative stress could be due to the other factors that have strong potential to destroy the anti-oxidant defense mechanism of BRCA1 wild type protein, thereby increase the susceptibility to breast cancer.

Several polymorphisms were reported in genes regulating one-carbon metabolism. Glutamate carboxypeptidase II (GCPII) C1561T (rs61886492) was reported to impair intestinal absorption of folate [13]. Reduced folate carrier 1 (RFC1) G80A (rs1051266) was found to impair the transport of folate across RBC membrane [14]. The functional relevance of cytosolic serine hydroxymethyltransferase (cSHMT) C1420T (rs1979277) polymorphism although not known, it was hypothesized that cSHMT polymorphism induces futile folate cycle in which SHMT and 5,10-methenyltetrahydrofolate synthetase enzymes buffer the intracellular concentration of 5-formyltetrahydrofolate to maintain one-carbon homeostasis [15]. Thymidylate synthase (TYMS) 5′-UTR 28 bp tandem repeat polymorphism was reported to affect transcription [16] while 3′-UTR ins6/del6 polymorphism was shown to affect mRNA stability [17]. Methylene tetrahydrofolate reductase (MTHFR) C677T (rs1801133) polymorphism was reported to induce thermolabile variant enzyme that has enhanced propensity to disassociate from active dimer form to inactive monomer form resulting in decreased specific activity of enzyme [18]. Methionine synthase (MTR) A2756G (rs1805087) and Methionine synthase reductase (MTRR) A66G (rs1801394) polymorphisms were shown to be associated with hyperhomocysteinemia, as these polymorphisms impair remethylation of homocysteine to methionine [19].

Based on the existing prima facie evidence supporting the association between markers of oxidative stress and one-carbon metabolism, the authors have explored the distribution of these eight putatively functional polymorphisms of one-carbon metabolism in sporadic breast cancer cases and controls; studied markers of oxidative stress (plasma 8-oxodG, homocysteine, and total glutathione) in sporadic breast cancer cases and controls, and established inter-relationships between these parameters.

## Materials and methods

### Study subjects

Eligible subjects were women with no family history of breast cancer or of any other cancer in the age group of 20–70 years. The authors have enrolled 222 newly diagnosed sporadic breast cancer cases and 235 age (±5 years)- and ethnicity-matched healthy controls at Nizam's Institute of Medical Sciences, Hyderabad, India before surgical or therapeutic intervention. The demographic characteristics of the studied subjects documented based on personal interview were: age (55.0 ± 13.0 vs. 54.6 ± 12.7 years), body mass index (27.3 ± 10.9 vs. 26.2 ± 6.8 kg/m$^2$), ethnic origin (Dravidian), age of menarche (13.4 ± 1.5 vs. 13.8 ± 1.8 years), age at the time of first full-term pregnancy (20.2 ± 5.2 vs. 20.8 ± 5.1 years), menopause status (78.9 vs. 78.9%), number of live births (2.6 ± 1.5 vs. 2.1 ± 1.4), history of breast feeding (94.7 vs. 97.4%), passive smoking (26.3 vs. 18.4%), and alcohol intake (5 vs. 0%). Subjects who are on any vitamin or anti-oxidant supplements and patients who had radiation and/or chemotherapy

were excluded from the study. This study was approved by the Institutional Ethical Committee of the Nizam's Institute of Medical Sciences, Hyderabad, India. Informed consent was obtained from the each subject.

For food frequency questionnaire (FFQ), all the subjects were given diaries to note the type of each food item, quantity taken, frequency (times per day/week/month/3 months or never) over a period of 2 weeks. As certain fruits and vegetables are seasonal, the availability of such food items was also considered while estimating their intake. Daily micronutrient intakes were calculated as grams of food multiplied by the amount of each nutrient in the food and the frequency of consumption, summing over all foods. The compositions of raw- and cooked-food items were determined from the 2007 reprint of Nutritive value of Indian foods [20]. In certain cases, where the information is not available on the composition, McCance and Widdowson's The composition of Foods [21] and the US Department of Agriculture's National Nutrient Database for Standard Reference release 19 (USDA, Washington, DC, USA) were consulted. The authors excluded the subjects with daily energy intake <650 kcal and >3,750 kcal and subjects who are on vitamin supplementation. The folate content derived from FFQ correlated strongly with plasma folate ($r = 0.26$, $P < 0.0001$).

### Sample collection

Whole blood samples were collected in commercially available EDTA vacutainers from all the eligible subjects at the time of interview. Plasma samples were separated immediately following centrifugation at $3300 \times g$/10 min at 4°C and stored in aliquots at −70°C until analysis.

### Estimation of biochemical parameters

Plasma 8-oxodG (Northwest Life Sciences specialties, USA) and estradiol (DRG International, Inc, Marburg, Germany) were measured using competitive ELISA kits as per the manufacturer's instructions. Ellman's method was used for the determination of glutathione [22]. Total plasma homocysteine was determined by using reverse phase HPLC method [23]. Plasma folate levels were estimated using Axsym folate kit (Abott Laboratories, USA).

### Genetic analysis

Genomic DNA was isolated from leukocytes using standard protocols [24]. PCR-RFLP method was used for the analysis of GCPII C1561T, RFC1 G80A, cSHMT C1420T, TYMS 3′-UTR ins6/del6, MTHFR C677T, MTR A2756G, and MTRR A66G polymorphisms. PCR-AFLP method was used for the analysis of TYMS 5′-UTR 28 bp tandem repeat polymorphism [23, 25]. The reaction conditions for genetic analysis are presented in Table 1.

### Statistical analysis

The Student's $t$ test was used to compare normally distributed continuous variables between breast cancer cases and controls. Linear regression was used to assess the association between the two given variables. ANOVA was used to compare the distribution of continuous variables across the three different genotypes. Fisher's exact test was used to calculate odds ratios (ORs) and confidence intervals (CIs). Unconditional logistic regression was used to obtain adjusted ORs by controlling for confounding effects namely age, body mass index, age of menarche, age at first full-term pregnancy, parity, and family history of breast cancer. All the statistical tests were 2-sided. A '$P$' value of <0.05 was taken to be significant. All analyses were performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

## Results

As illustrated in Table 2, RFC1 G80A and MTHFR C677T were associated with increased risks of 1.34 (95% CI 1.01–1.79)- and 1.84 (95% CI 1.14–3.00)-folds for sporadic breast cancer while cSHMT C1420T was associated with reduced risk (OR 0.71, 95% CI 0.53–0.94).

Results, as shown in Fig. 1, indicate increased plasma 8-oxodG (mean ± SE 5.59 ± 0.60 vs. 3.50 ± 0.40 ng/ml, $P < 0.004$) and total plasma homocysteine (16.07 ± 0.53 vs. 12.45 ± 0.98 μmol/l. $P < 0.0001$), and decreased glutathione (379.6 ± 45.82 vs. 500.6 ± 11.46 μmol/l, $P < 0.01$) levels in breast cancer cases as compared to the controls. Plasma folate levels in sporadic breast cancer cases were lower than healthy controls (Mean ± SD: 6.84 ± 1.27 ng/ml vs. 7.10 ± 1.27 ng/ml, $P = 0.03$). Results also show increased levels of plasma estradiol (129.8 ± 10.19 vs. 108.4 ± 12.25 pg/ml, $P = 0.18$) in breast cancer cases, but this increase is not statistically significant (Fig 1). The results of the study also show that the dietary folate intake (340.5 ± 7.82 vs. 371.6 ± 8.21 μg/day, $P = 0.006$) was lower in cases than in controls.

Spearman rank correlation was done to assess the association of the plasma 8-oxodG with plasma glutathione, homocysteine, and dietary folate. Results indicate decrease in oxidative DNA damage with the increase in plasma glutathione ($r = -0.15$, $P < 0.001$), dietary folate ($r = -0.26$, $P < 0.0001$), and plasma folate ($r = -0.25$, $P < 0.0001$). Results also show increase in oxidative DNA

**Table 1** Reaction conditions for PCR-based genetic analysis

| Polymorphism | Primers (5′……3′) | PCR conditions | Restriction enzyme | Restriction site |
|---|---|---|---|---|
| GCP II C1561T | CAT TCT GGT AGG AAT TTA GCA | D:95°/30 s | Acc I | +(T allele) |
| ×35 | AAA CAC CAC CTA TGT TTA ACA | A:50°/30 s | | |
| | | E:72°/30 s | | |
| RFC G80A | AGT GTC ACC TTC GTC CCC TC | D:95°/1 min | Hha I | −(A allele) |
| ×30 | CTC CCG CGT GAA GTT CTT- | A:59°/1 min | | |
| | | E:72°/1 min | | |
| cSHMT C1420T | GTG TGG GGT GAC TTC ATT TGT G | D:95°/30 s | Ear I | +(C allele) |
| ×35 | GGA GCA GCT CAT CCA TCT CTC | A:56°/30 s | | |
| | | E:72°/30 s | | |
| TYMS 5′-UTR (AFLP) | CGT GGC TCC TGC GTT TCC | D:95°/30 s | AFLP | AFLP |
| ×35 | GAG CCG GCC ACA GGC AT | A:62°/30 s | | |
| | | E:72°/45 s | | |
| TYMS 3′-UTR | CAAATCTGAGGGAGCTGAGT | D:95°/30 s | DraI | −(del6) |
| Ins6/del6 | CAGATAAGTGGCAGTACAGA | A:58°/45 s | | |
| | | E:72°/45 s | | |
| MTHFR C677T | TTT GAG GCT GAC CTG AAG CAC TTG AAG GAG | D:95°/1 min | Hinf I | +(T allele) |
| ×30 | GAG TGG TAG CCC TGG ATG GGA AAG ATC CCG | A:60°/1 min | | |
| | | E:72°/1 min | | |
| MTR A2756G | TGT TCC CAG CTG TTA GAT GAA AAT C | D:95°/30 s | Hae III | +(G allele) |
| ×40 | GAT CCA AAG CCT TTT ACA CTC CTC | A:60°/30 s | | |
| | | E:72°/30 s | | |
| MTRR A66G | GCA AAG GCC ATC GCA GAA GAC AT | D:95°/1 min | Nde I | +(A allele) |
| ×30 | GTG AAG ATC TGC AGA AAA TCC ATG TA | A:55°/30 s | | |
| | | E:72°/30 s | | |

*D* denaturation, *A* annealing, *E* extension, × number of cycles. + restriction site created, − restriction site abolished

damage (8-oxodG) with the increase in total plasma homocysteine ($r = 0.27$, $P < 0.0001$).

Results, as shown in Fig. 2, indicate increase in oxidative DNA damage in post-menopausal women than in pre-menopausal women ($P = 0.02$). Among the eight putatively functional polymorphisms studied, only two polymorphisms were found to influence the plasma 8-oxodG levels. RFC1 G80A polymorphism was associated with increased oxidative stress ($P = 0.04$), while cSHMT C1420T was associated with reduced oxidative stress ($P < 0.05$) (Fig. 2).

## Discussion

The results of this study show that RFC1 G80A, and MTHFR C677T are independent risk factors for sporadic breast cancer, while cSHMT confers protection. Previous studies have demonstrated that in subjects with low dietary/plasma folate, MTHFR C677T is associated with breast cancer risk [26, 27]. RFC1 G80A showed no association with breast cancer in a study by Xu et al. [28]. The protective role of cSHMT C1420T was also documented in a study on Chinese population [29]. This study is the first to investigate the role of GCPII C1561T polymorphism in breast cancer risk. Existing studies show no association between TYMS 5′-UTR and 3′-UTR polymorphisms and breast cancer, consistent with the observation in this study [30, 31]. The association studies on MTR A2756G were inconsistent with one group showing reduced breast cancer risk [32] while two studies reporting null results [33, 34]. MTRR A66G polymorphism showed null association in several studies [33, 34]. This study demonstrated null association for MTR A2756G and MTRR A66G polymorphisms. Meta-analysis by Lewis et al. on MTHFR C677T showed no association of this polymorphism while indicating protective role of folate in reducing the breast cancer risk [35]. The association studies showed great variation across different populations and ethnic groups as evidenced by the epidemiological review by Xu et al. [36].

In this study, the authors examined, for the first time, markers of oxidative stress in plasma specimens of women with sporadic breast cancer and healthy controls. The authors demonstrate increased plasma 8-oxodG and homocysteine, and decreased glutathione and dietary folate in

**Table 2** Distribution of eight functional polymorphisms in sporadic breast cancer cases and controls

| Polymorphism | WW | WM | MM | MAF (%) | Adjusted OR (95% CI) | *P* value |
|---|---|---|---|---|---|---|
| GCPII C1561T | CC | CT | TT | T-allele | | |
| Cases | 187 | 35 | 0 | 35/444 (7.9%) | 0.83 (0.49–1.40) | 0.83 |
| Controls | 188 | 47 | 0 | 47/470 (10%) | | |
| RFC1 G80A | GG | GA | AA | A-allele | | |
| Cases | 81 | 95 | 46 | 187/444 (42.1%) | **1.34 (1.01–1.79)** | **0.04** |
| Controls | 93 | 120 | 22 | 164/470 (34.9%) | | |
| cSHMT C1420T | CC | CT | TT | T-allele | | |
| Cases | 53 | 116 | 53 | 222/444 (50.0%) | **0.71 (0.53–0.94)** | **0.02** |
| Controls | 43 | 113 | 79 | 271/470 (57.7%) | | |
| TYMS 5′-UTR | 3R3R | 2R3R | 2R2R | 2R-allele | | |
| Cases | 90 | 102 | 30 | 162/444 (36.5%) | 1.04 (0.77–1.41) | 0.79 |
| Controls | 97 | 113 | 25 | 163/470 (34.7%) | | |
| TYMS 3′-UTR | Ins6/ins6 | Del6/Ins6 | Del6/Del6 | Del6-allele | | |
| Cases | 52 | 110 | 60 | 230/444 (51.8%) | 1.06 (0.79–1.42) | 0.70 |
| Controls | 57 | 126 | 52 | 230/470 (48.9%) | | |
| MTHFR C677T | CC | CT | TT | T-allele | | |
| Cases | 168 | 53 | 1 | 55/444 (12.4%) | **1.84 (1.14–3.00)** | **0.01** |
| Controls | 198 | 37 | 0 | 37/470 (7.9%) | | |
| MTR A2756G | AA | AG | GG | G-allele | | |
| Cases | 109 | 102 | 11 | 124/444 (27.9%) | 1.06 (0.76–1.49) | 0.72 |
| Controls | 116 | 109 | 10 | 129/470 (27.4%) | | |
| MTRR A66G | GG | GA | AA | A-allele | | |
| Cases | 55 | 156 | 11 | 178/444 (40.0%) | 1.40 (0.95–2.04) | 0.08 |
| Controls | 73 | 150 | 12 | 174/470 (37.0%) | | |

*WW* wild genotype, *WM* heterozygous genotype, *MM* homozygous mutant genotype, *MAF* minor allele frequency, *Adjusted OR* odds ratio adjusted for confounding variables such as age, body mass index, age of menarche, menopause status and parity, all the bold letters indicate significant associations

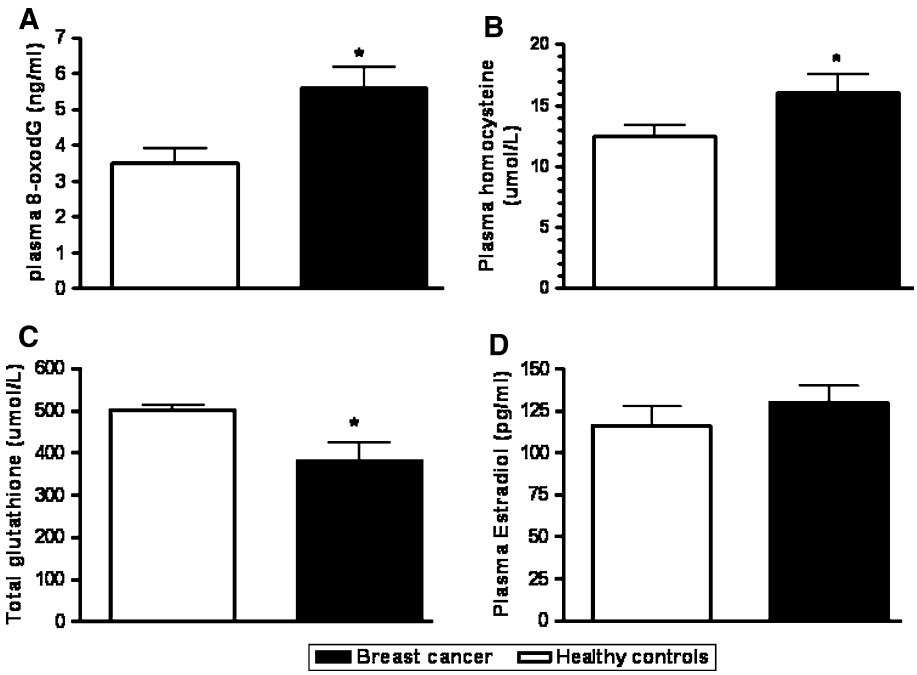



**Fig. 1** Plasma levels of **a** 8-oxo-2′-deoxyguanosine, **b** homocysteine **c** total glutathione, and **d** estradiol in healthy controls and breast cancer cases. Results show significant increase in plasma 8-oxo-2-deoxyguanosine, and homocysteine, and significant decrease in glutathione levels in breast cancer cases

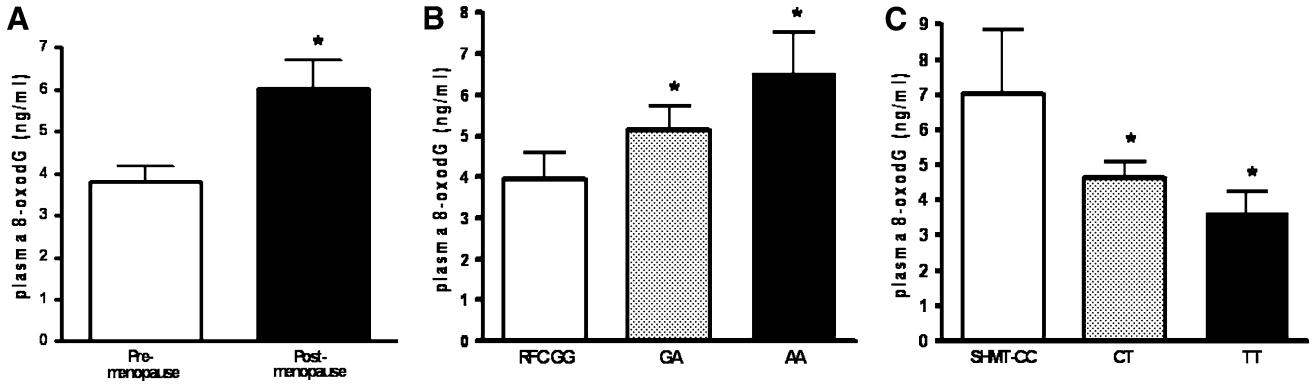



**Fig. 2** Analysis of variance (ANOVA) between the **a** 8-oxo-2-deoxyguanosine and pre-and post menopausal, **b** 8-oxo-2-deoxyguanosine and RFC GG, RFC GA, RFC AA, **c** 8-oxo-2-deoxyguanosine and SHMT CC, SHMT CT, and SHMT TT. Results show increase in oxidative DNA damage with the increase in the age of menarche. Post-menopausal women demonstrated higher oxidative stress than the pre-menopausal women. RFC1 G80A polymorphism was associated with the increased oxidative stress, while cSHMT C1420T was associated with reduced oxidative stress

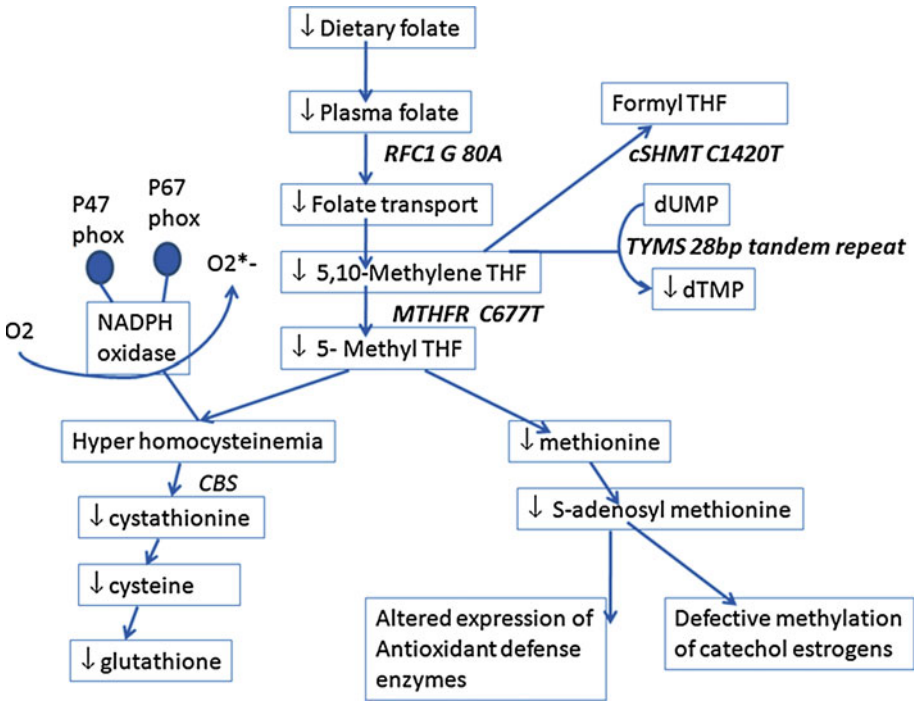
the sporadic breast cancer cases compared with controls indicating oxidative stress (Fig. 1). This observation is consistent with the other studies conducted on breast tissue, leukocytes, and urine specimens [5, 6]. Previous studies concerning the oxidative stress in breast cancer were focused on hereditary breast cancer, specifically on BRCA1-mutant women [37], whereas no studies were specifically focused on sporadic breast cancer. A recent study has demonstrated the clinical utility of 8-oxodG as prognostic marker for breast cancer [38].

Oxidative stress in cancer was suggested to mediate through extensive granulocyte activation, inflammatory cytokines, and malignant cells producing excessive ROS [39–41]. These three conditions are characteristic for advanced stages of cancer development. In this study, all the cases had low or intermediate grade breast cancer, and these mechanisms may not explain oxidative stress in sporadic breast cancer completely. In order to evaluate the other possible factors that contribute to oxidative stress, the authors correlated 8-oxodG with different parameters. Glutathione, dietary folate, and estradiol were associated with decreased oxidative stress while homocysteine was associated with increased oxidative stress. The inverse association between the gluatathione and oxidative stress is well documented. Glutathione is known to conjugate with electrophiles (Phase II) during biotransformation and thus a potential scavenger of free radicals. Folate is essential for the synthesis, repair, and methylation of DNA as well as methylation of catechol estrogens to methoxy estrogens. The deficiency of folate perturbs these crucial biochemical processes thus explaining the association with oxidative stress. The animal studies support these observations [7, 8].

Estradiol is the precursor for catechol estrogen as well as for methoxyestrogen. When the methyl group availability is low due to RFC1 G80A and MTHFR C677T polymorphisms, it might act as pro-oxidant mediated through catechol estrogen. When the methyl group availability is adequate as in the case of cSHMT C1420T carriers, it acts as anti-oxidant mediated through methoxy estrogen. The strong association between the hyperhomocysteinemia and oxidative DNA damage substantiates findings of an earlier study by the authors showing the dose-dependent association between the homocysteine and DNA damage in vivo and in vitro [42]. The association of homocysteine with oxidative DNA damage is probably mediated through the superoxide generation as evidenced by the study of Oikawa et al. indicating increase in 8-oxodG with the increase in homocysteine in human leukemia cell line HL-60 and no such increase in hydrogen peroxide-resistant clone HP100 [43]. They further demonstrated that the mild increase in homocysteine (20 μM) induces piperidine-labile sites at the thymine residues and moderate-to-severe increase (100 μM) results in DNA damage at guanine residues [43].

In order to establish the association of oxidative DNA damage with physiological and genetic variants, all the demographic and genetic variables were correlated with 8-oxodG. The authors observed increased oxidative stress in post-menopausal women. The increased levels of 8-oxodG in post-menopausal women can be attributed to hormonal changes at the time of menopause and only source of estrogen production being the peripheral estrogen synthesis.

Among the eight putatively functional polymorphisms in one-carbon metabolism, two polymorphisms, i.e., RFC1 G80A and cSHMT C1420T were observed to influence oxidative DNA damage. RFC1 G80A polymorphism was associated with increased oxidative DNA damage, while cSHMT C1420T polymorphism was associated with decreased oxidative DNA damage (Fig 2). In a recent study,




**Scheme 1** Mechanism of oxidative stress induced by aberration in one-carbon metabolism. *RFC1* reduced folate carrier 1, *cSHMT* cytosolic serine hydroxymethyl transferase, *TYMS* thymidylate synthase, *THF* tetrahydrofolate, *dUMP* deoxyribo uracil monophosphate, *dTMP* deoxyribo thymine monophosphate, *MTHFR* methylene tetrahydrofolate reductase, *CBS* cystathionine beta synthase

the authors demonstrated low plasma folate levels in subjects carrying RFC1 80A-variant allele and high plasma folate in subjects carrying cSHMT 1420 T-variant allele. RFC1 maintains intracellular folate levels under physiological conditions [14]. Under the conditions of folate deprivation, RFC1 was reported to down-regulate as an adaptive response and lead to severe RBC folate deficiency [44]. cSHMT carries out reversible conversion of tetrahydrofolate to 5,10-methylene tetrahydrofolate (by accepting one-carbon from serine) and irreversible conversion of 5,10-methylene tetrahydrofolate to 5-formyl tetrahydrofolate (futile folate cycle). Formation of 5-formyl tetrahydrofolate helps in maintaining one-carbon homeostasis during the rapidly proliferative stages of development [45]. The mechanism of induction of oxidative DNA damage by RFC1 G80A is probably mediated through RBC folate deficiency. The protection conferred by cSHMT is probably due to increase in one-carbon moieties that negative the effect of ROS. The lack of association between MTHFR C677T and 8-oxodG was also reported by Dorszewska et al. [46], which could be due to no direct influence of this polymorphism on plasma or red blood cell folate. The risk attributed by MTHFR C677T polymorphism could be due to other alternative mechanisms, specifically through aberrant DNA methylation, as the product of MTHFR catalysis i.e., 5-methyl tetrahydrofolate is essential for the synthesis of SAM.

All the parameters observed to be associated with oxidative DNA damage have well-established inter-relationships. Deficiency of dietary folate influences plasma folate while RFC1 G80A influences RBC folate. cSHMT increases plasma folate pool by induction of futile folate cycle. Deficiency of plasma folate or RBC folate is associated with the elevation of homocysteine. The mechanism of oxidative stress induced by these parameters could be mediated through increased DNA damage, decreased DNA methylation, or decreased methylation of catechol estrogens (Scheme 1). Further accumulation of homocysteine prevents the events in trans-sulfuration pathway necessary for the synthesis of anti-oxidant glutathione, which in turn might perturbate the delicate balance between pro-oxidants and anti-oxidants. Determining the end points such as markers modulated by SAM and interactions with polymorphisms in Phase I and Phase II enzymes of xenobiotic metabolism will substantiate these findings further. Further studies focusing on 8-oxodG content in leukocyte DNA and urine simultaneously might be helpful in distinguishing the rates of oxidative DNA damage and repair.

To conclude, this study suggests that low dietary folate, RFC1 G80A, and MTHFR C677T are independent risk factors for sporadic breast cancer and they induce oxidative stress by affecting the folate pool or by increasing homocysteine. The observation showing inverse association between folate and oxidative stress, if translated to a clinical setting, might prove as a good preventive strategy specifically in reducing oxidative stress and breast cancer risk to some extent.

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