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ORIGINAL ARTICLE

# Expression deregulation of DNA repair pathway genes in gastric cancer

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**Abstract**

This study was designed to check correlation of mRNA and protein expression of BER pathway genes(*XRCC1, OGG1)* and a proliferation marker (*Ki-67)* in 100 gastric tissue samples and controls (adjacent uninvolved area). The expression was estimated *using* real time PCR and immunohistochemistry. Genomic instability was also calculated in the same study cohort using 8-OHdG assay, DNA fragmentation assay and comet assay. A significant downregulation of *XRCC1* ($p < 0.0001$) and *OGG1* ($p < 0.0001$) expression was observed in gastric cancer tumors vs controls. When analyzed with spearman correlation, significant positive correlation was observed between *OGG1* vs *XRCC1* ($r = 0.319^{*}$, $p < 0.02$) and significant negative correlation was observed between *OGG1* vs *Ki-67* ($r = -0.462^{**}$, $p < 0.001$) and *XRCC1* vs *Ki-67* ($r = -0.589^{**}$, $p < 0.001$) in gastric cancer tumors. Significantly higher level of 8-OHdG, when compared to controls, was observed in gastric cancer tumors ($p < 0.0001$). DNA fragmentation assay and comet assay showed the formation of increased ladder patterns and comets in gastric cancer tumors when compared with controls These findings suggest that dysregulation of *XRCC1, OGG1* combined with overexpression of *Ki-67* may contribute to progression of gastric cancer and may help to sub-classify patients within diverse risk groups for therapeutic advantages.

**Keywords** BER pathway, Gastric cancer, XRCC1, OGG1, DNA fragmentation, Proliferation.



## Introduction

Oxidative DNA damage and DNA repair mediate the development of several human pathologies, including gastric cancer. The major pathway for oxidative DNA damage repair is base excision repair (BER) pathway [1]. Base excision repair (BER) is the primary guardian pathway against damage that results from cellular metabolism, including reactive oxygen species, methylation, deamination and hydroxylation. Therefore, base excision repair is a universal event in cells and is relevant for preventing mutagenesis [2]. 8-oxoguanine DNA glycosylase (*OGG1*) and X-rays repair cross-complementing 1 (*XRCC1*) are important players of this pathway.

*XRCC1* encodes a scaffolding protein that interacts with and modulates DNA ligase III, polymerase beta and poly (ADP-ribose) polymerase to efficiently carry out base excision repair pathway [3]. Cells deficient in *XRCC1* exhibit increased sensitivity towards DNA damaging agents such as ROS and ionizing radiation and hence impair repair capacity. Expression dysregulations of this gene has been reported in various cancers such as head and neck squamous cell carcinoma (HNSCC) [4,5], colorectal carcinoma [6], cervical cancer [7] and breast cancer [8].

*OGG1* gene is involved in BER pathway implicating maintenance of genome and preventing oncogenesis. It is a major glycosylase with strong specificity to 8-oxoG and formamidopyrimidines lesions [9]. Several studies have reported the association of *OGG1* polymorphisms with various cancers such as bladder cancer [10], ovarian cancer [11], laryngeal cancer [12], and colorectal carcinoma [13]. Aberrant mRNA expression of this gene has also been reported in different cancers [14,12,15], evidencing its functional importance.

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Fewer studies have examined the direct association between these genes and gastric carcinogenesis. Current study was designed to assess the associations between expression variation of these BER pathway genes and proliferation marker, *Ki-67,* with gastric cancer Moreover, correlation of expression profiling of these selected genes with genomic instability in gastric cancer samples was also studied.

## Material and methods

### Publication search

To identify the relevant literature, a PubMed search was performed for *XRCC1* and *OGG1* studies. The following criteria were adopted for the inclusion of studies in this systematic review:

(i) studies on mutation screening and expression analysis of *XRCC1* and *OGG1* in patients with gastric cancer;
(ii) studies using blood samples, gastric tumor samples, control blood samples and adjacent noncancerous tissues, respectively, for comparison;
(iii) studies published as full articles in English.

Studies using cancer cell lines, serum or saliva samples were not included. Review articles and meta-analyses were also excluded from the present study.

### Tumor sample collection

A total of 100 gastrectomy samples (either total, subtotal or partial) were involved in the current study. The surgeries were performed at the surgical units of Holy Family Hospital, Rawalpindi, Pakistan. The specific tumor core, invasive margins and adjacent healthy mucosa were obtained from these surgically removed tissues and directly stored for RNA later at −80 °C. Tissues were subjected to cryo-sectioning and stained with hematoxylin and eosin (H/E). Stained slides were then examined by consultant histopathologist for presence of cancerous cells. While uninvolved healthy tissue, at 2 cm away from the tumor was utilized as controls. A specifically designed questionnaire was filled by each patient to ensure the voluntary participation. Information regarding age, gender and other relevant clinical data was also recorded and is summarized in Table 1. The procedure and methodological aspects of the current study were previously approved by the institutional ethical review board of COMSATS University (CUI), Islamabad and collaborating hospitals.

### RNA extraction and cDNA synthesis

Trizol method was used for the isolation of RNA from tumor and control tissues. The integrity of RNA was visualized using 1% TAE gel. Quantification of the yielded RNA was performed by UV Spectrophotometer using wavelengths of 260 nm and 280 nm, followed by storage at −80 °C. The cDNA was synthesized by SuperScript III First Strand Synthesis system (Invitrogen) using extracted RNA and stored at 4 °C for further use.

**Table 1** Demographic and clinical parameters of the study cohort.

| Variables | Cancer Patients |
|---|---|
| **Age (years±S.D)** | |
| Mean | 53 ± 0.5 |
| <53 | 44 |
| >53 | 56 |
| **Gender (n)** | |
| Male | 62 |
| Female | 38 |
| **Types of tumor (n)** | |
| Adeno-carcinoma | 74 |
| Signet Ring Cell Carcinoma | 10 |
| GIST | 8 |
| Neuroendocrine Tumor | 8 |
| **Clinical Staging (n)** | |
| I-II | 76 |
| III-IV | 24 |
| **T Staging (n)** | |
| T1-T2 | 62 |
| T3-T4 | 38 |
| **N staging (n)** | |
| N0 n (%) | 50 |
| N1-N2 n (%) | 50 |
| **M Staging (n)** | |
| M0 n (%) | 82 |
| M1 n (%) | 18 |
| **Survival Data (n)** | |
| Cured n (%) | 50 |
| Under Treatment n (%) | 40 |
| Deceased n (%) | 10 |

**Table 2** Real Time based primer sequence of *XRCC1* and *OGG1* genes.

| Primer Name | Sequence | Product Size |
|---|---|---|
| XRCC1 F | GTGGCAGCGGAGATGAAG | 133bp |
| XRCC1 R | ACTGGTGAGGCTGCTTTG | |
| OGG1 F | TCAGGAAAGCCGGAGAATTG | 120bp |
| OGG1 R | CCCACACGGTGCTGTTTA | |

### Quantitative polymerase chain reaction (q-PCR)

The primers specific for all respective genes (including the internal control, $\beta$*-actin*) were designed (Table 2) and optimized using integrated DNA technology (IDT) USA. The qPCR reaction mixture was comprised of 5 µl of Syber green master mix, 2 µl of RNase free water and 1 µl of cDNA, forward and reverse primer each in a final volume of 10 µl. The qPCR was performed using Applied Biosystems Step 1 plus PCR system with primer specific thermocycler conditions. The relative mRNA expression of respective genes was computed using $2^{-\Delta\Delta Ct}$ analysis method with $\beta$*-actin* as reference gene.

### Immunohistochemistry

Expression of *XRCC1, OGG1* and *Ki-67* at translational level was analyzed by immuno-histochemical staining using 100

gastric cancer samples along with adjacent non-cancerous tissues taken as controls. Immuno-histochemical analysis was performed using the DAB chromogen staining kit (Sigma) as described previously [16].

Both tumor and control tissue slides were incubated with mouse anti-*XRCC1* (Santa Cruz Biotechnology, Inc. UK), anti-*OGG1* (Novus Biologicals, Inc. USA) and anti-*Ki-67* (Novus Biologicals, Inc. USA) of 1:500 and 1: 1000 dilutions respectively for 1 h at room temperature. Both positive and negative controls were used to validate the procedure and specificity of primary and secondary antibody. Ductal carcinoma of breast served as positive control for *OGG1* and *XRCC1* while tonsil tissue was used as positive control for *Ki-67*. For negative controls the procedure remained same except for the primary antibody, where phosphate buffer saline (PBS) was added instead.

Three independent histo-pathologists, unaware of the clinical data evaluated the immuno-histochemical reactions using light microscopy and the relative intensities. Any disagreement on results was ruled out by mutual consent of the histopathologists.

### Scoring criteria

At least ten high power fields were scanned to randomly count the tumor cells and evaluate the immunoreactivity by the formula:

$$\text{Immunoreactive score} = \text{intensity score} \times \text{proportion score}$$

Immunoreactivity was scaled on a spectrum of score values from 0 to 12.1.0–4 denotes low immunoreactivity and $>4$ denotes high immunoreactivity.

Intensity was graded from 0 to 3 with negative to strong staining. Intensity score was specified into three levels ranging from (0–3); 0 for negative intensity, 1 for weak intensity, 2 for moderate intensity and 3 for strong staining intensity.

If there was not a single positive cell the proportion score was valued as 0. Similarly, an average of $\leq$10%, 11–50%, 51–80% and $>$ 80% positive cells distribution earned the score of 1, 2, 3 and 4 respectively.

## Measurement of 8-OHdG level in study cohort

8-OHdG level was measured in tissue lysate of gastric cancer tissues and adjacent control tissues. Snap frozen tissue samples were homogenized in sonicator, after addition of 5 ml homogenizer buffer (0.1 M phosphate buffer containing 1 mM of EDTA at pH 7.4). After homogenization, samples were centrifuged for 10 min and supernatant was decanted, purified and processed further.

DNA was then digested using nuclease P1(Sigma), followed by addition of 1 unit of alkaline phosphatase per 100 µg of DNA. The samples were incubated at 37 °C for 30 min. Samples were then boiled for 10 min and placed on ice until use. 8-OHdG level of respective samples was measured with commercially available 8–hydroxy 2 deoxyguanosine ELISA kit (abcam) as per manufacturer's provided protocol.

## DNA fragmentation assay

DNA damage in gastric tissue samples and adjacent control tissues was assessed by DNA fragmentation assay. The protocol adopted was modified version as given by Ahmed et al., [17]. Gastric cancer tissue samples and controls were lysed in lysis buffer (50 M Tris-HCl, pH 8 + 10 mM EDTA, pH 8 + 100 mM NaCl + 1% SDS) and 15 µl of proteinase K solution (20 µg/ml) and incubated overnight at 55 °C. DNA was extracted using phenol chloroform method and extracted DNA was dissolved in an appropriate volume of 1 x TE buffer. After RNAse (40 µg/ml) incubation for 2 h at 37 °C, the samples were applied and analyzed on agarose (1.5%) gel with ethidium bromide (0.5 µg/ml).

## Comet assay

Procedure for alkaline comet assay used in present studies was in accordance with the method described by Akram et al., (2018) with minor modifications [18]. Gastric cancer tissues and adjacent control tissues were homogenized in saline solution and processed for comet assay to detect the overall DNA damage in gastric tissue and adjacent section (taken as controls). Three layered procedure was used and samples were sandwiched between first and second layer of LMP agarose gel. 50 comets were scored for each sample using fluorescent microscope (Leica) equipped with filters and digital cameras. Comets were analyzed using Casp-Labsoftware**.**

## Statistical analysis

Association of *XRCC1, OGG1* and *Ki-67* expression levels with the clinical and histopathological parameters (e.g. TNM and grade) was determined using $\chi$2-test, one-way analysis of variance (ANOVA) and Tukey s' post hoc test. Furthermore, the spearman correlation coefficient was used to assess correlations among the gene expression and clinical and histopathological parameters. Receiver Operating curves (ROC) were generated and area under ROC curve was calculated to evaluate the diagnostic value of *XRCC1* and *OGG1* expression level in discriminating tumor and non-tumor states of the samples. Statistical representation of the data was carried out using GraphPad Prism5 software and SPSS 6.0 software package.

# Results

## Study characteristics

A total of 126 relevant studies were retrieved after a comprehensive search of the PubMed database, published between 2005 and 2018. Of the selected studies, 88 studies were excluded for being not relevant to gastric cancer or *XRCC1* and *OGG1* and 18 studies (4 meta-analyses, 3 review, 10 studies carried out on cell lines and 1 study in language other than English) were also excluded from present systematic review. Resultantly, 20 studies were included in the systematic review, details of which are listed in Supplementary Table 1.

## Expression analysis

### XRCC1

In this study 100 gastric cancer tissue samples along with adjacent noncancerous tissue samples taken as controls were used. The demographic characteristics of the study cohort are shown in Table 1. Relative expression of *XRCC1* was observed in gastric cancer tumors along with adjacent normal tissues used as controls. A significant ($p < 0.0001$) downregulated expression of *XRCC1* gene was observed in GC tumors compared to controls as shown in Fig. 1A. Relative expression of *XRCC1* mRNA was analyzed in different clinical stages of gastric cancer. A significant down regulation was observed in advanced clinical stages (stage III and IV; $p < 0.01$) and in advanced T-stage (T3-T4; $p < 0.014$) carcinomas compared to early clinical stage (stage I and II) and early T-stage (T1-T2) of gastric carcinoma (Fig. 1B). Relative expression of *XRCC1* mRNA was also analyzed in different N and M stages of gastric cancer patients. A significant down regulation of *XRCC1* mRNA was observed in N2-N3 stages ($p < 0.0009$) and in M1 stage ($p < 0.0001$) when compared to N0–N1 and M0. This down regulation is shown in Fig. 1B.

Expression level of XRCC1 gene was also compared for tumor types and survival levels A significant downregulation of *XRCC1* gene was observed in different anatomical sites of GC such as adenocarcinoma, signet ring cell carcinoma and GIST ($p < 0.001$) compared to neuroendocrine tumors (Fig. 1C). Similar trend of downregulation was observed in deceased GC patients ($p < 0.01$) compared to cured and under treatment GC patients as shown in Fig. 1C.

### OGG1

Expression levels of second selected BER pathway gene, *OGG1* was also evaluated and significant downregulated expression of the said gene ($p < 0.0001$) was observed in tumors compared to control tissues (Fig. 1D). Expression levels of *OGG1* was further analyzed in different histopathological parameters of GC such as clinical stages and TNM stages as shown in Fig. 1E.

Downregulated expression of *OGG1* gene was observed in advanced clinical stages vs early clinical stages ($p < 0.0009$), advanced T-stages vs early T-stages ($p < 0.0278$), advance N-stages vs early N-stages ($p < 0.0012$) and advance M-stage vs early M-stage ($p < 0.0006$) of GC tumors (Fig. 1E). Expression level of *OGG1* gene was compared for different anatomical sites and survival status of GC patients, as shown in Fig. 1F. A significant downregulation of *OGG1* was observed in deceased and under treatment GC patients ($p < 0.01$) compared to cured GC patients (Fig. 1F).

In this study, significant upregulation of proliferation marker, *Ki-67* was also observed in GC tumors compared to controls as shown in Fig. 1G. Further analysis showed the significant upregulation of *Ki-67* in advanced clinical stages vs early clinical stages ($p < 0.04$) and in advance N-stages vs early N-stage ($p < 0.0207$) in GC patients (Fig. 1H). Expression level of *Ki-67* was also evaluated for anatomical sites and survival status but no significant difference in upregulation of *Ki-67* was observed, as shown in Fig. 1I.

## Protein expression analysis of selected genes using IHC

Protein expression analysis of *XRCC1, OGG1* and *Ki-67* was observed in tumor tissues along with adjacent normal tissue. The IHC analysis resulted in a varied yet significant pattern of expression. The cut off value for immunostaining to be termed positive was more than 10% of tumor cells showing distinct staining and 25% in case of *Ki 67*. A downregulated expression of *XRCC1* and *OGG1* was observed in tumors compared to controls as shown in Fig. 2A-2D.

### XRCC1

When examined, 82% tumors displayed downregulated expression and 18% upregulated expression. Further analysis showed that immunoreactive score of protein was observed significantly lower in tumors ($p < 0.0001$) compared to controls (Fig. 3A). The relative intensities showed that 57% tumors exhibited weak immunoreactive intensities, 33% moderate immunoreactive intensities and 10% tumors showed strong immunoreactive intensities. The relative intensities also showed that weak immunoreactive intensity ($p < 0.0001$) was significantly more prevalent in N-stage tumors compared to moderate and strong immunoreactive intensity (Fig. 3B).

### OGG1

In the case of IHC staining for *OGG1,*79% tumors showed downregulated expression and 21% upregulated expression, respectively in gastric cancer patients. The calculated immunoreactive score of *OGG1* genes was observed significantly lower in gastric cancer tumors ($p < 0.0001$) compared to controls as shown in Fig. 3C. When the relative intensities were analyzed, 63% tumors showed weak immunoreactive intensities, 25% moderate immunoreactive intensities and 12% tumors showed strong immunoreactive intensities. Weak immunoreactive intensity was observed significantly lower in T-stage ($p < 0.04$) and N-stage ($p < 0.0009$) compared to moderate and strong immunoreactive intensities (Fig. 3D).

In case of proliferation marker *Ki-67*, upregulated expression of *Ki-67* was observed in tumor samples compared to controls (Fig. 2E and 2F). When examined, 89% tumors showed upregulated expression and 11% showed downregulated expression. The immunoreactive score of *Ki-67* was observed significantly higher in tumors ($p < 0.0001$) compared to controls as shown in Fig. 3E. The relative intensities showed that 63% tumors showed strong immunoreactive intensity, 23% tumors moderate immunoreactive intensities and 14% tumors showed strong immunoreactive intensities. Further analysis showed that weak immunoreactive intensity ($p < 0.04$) was significantly higher in N-stage compared to moderate and strong immunoreactive intensity, as shown in Fig. 3F.

## Correlations between BER pathway genes (*OGG1, XRCC1*) and proliferation marker (*Ki-67*)

In case of clinicopathological-clinicopathological characteristic relationship, a positive correlation was observed (Table 3) between clinical-stage vs T-stage ($r = 0.905^{**}$, $p < 0.0001$), clinical-stage vs N-stage ($r = 0.778^{**}$, $p < 0.0001$), clinical-stage vs M-stage ($r = 0.637^{**}$, $p < 0.0001$), T-stage vs N-stage

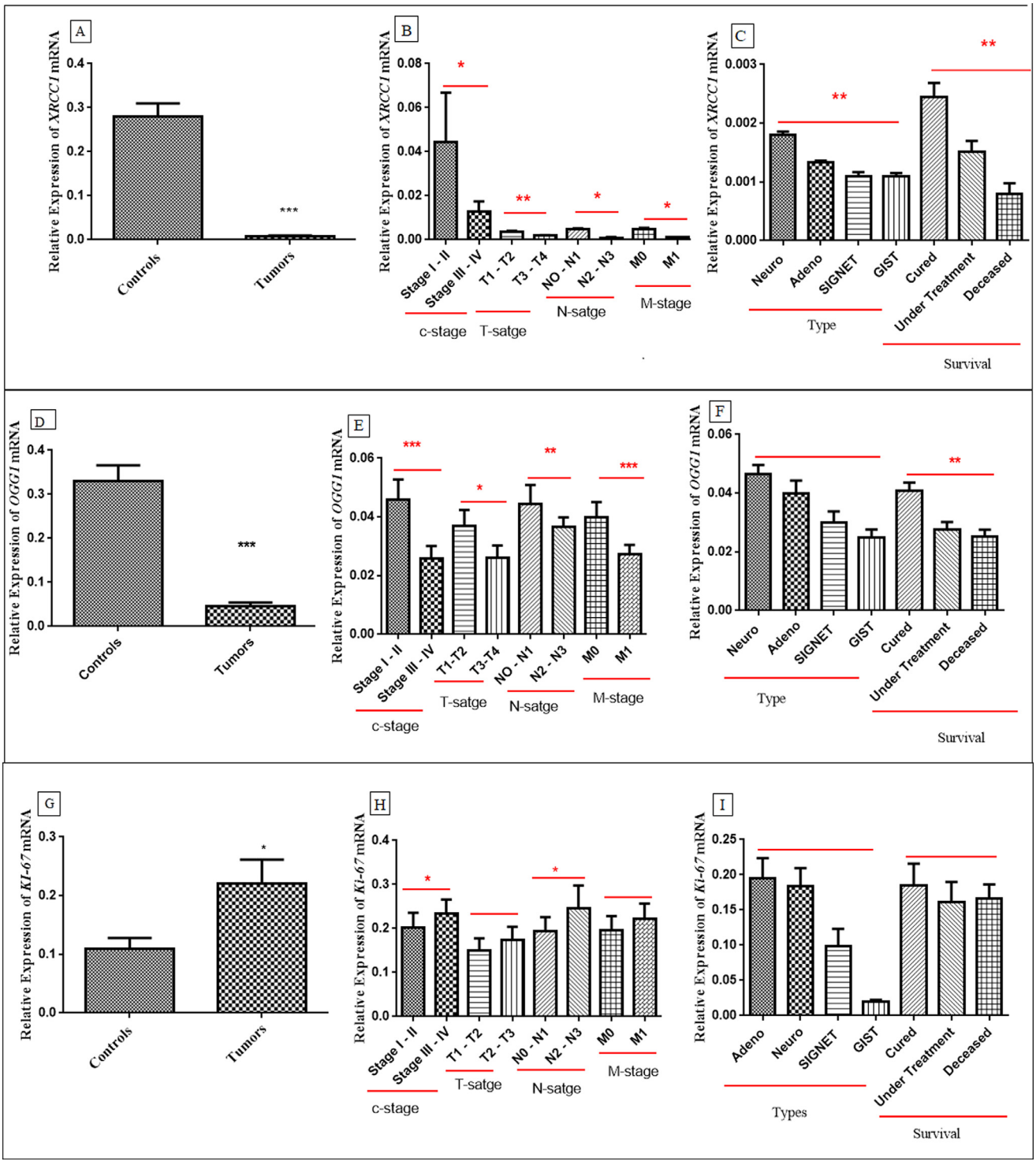



**Fig. 1** mRNA expression of (A) *XRCC1* in gastric cancer tumor samples and normal control samples, (B) *XRCC1* in gastric tumor samples with clinical stage and TNM (C) *XRCC1* in gastric tumor samples with different anatomical sites of gastric region and gastric tumor samples with different survival status. (D) *OGG1* in gastric cancer tumor samples and normal control samples, (E) *OGG1* in gastric tumor samples with clinical stage and TNM (F) *OGG1* in gastric tumor samples with different anatomical sites of gastric region and gastric tumor samples with different survival status. (G) *Ki-67* in gastric cancer tumor samples and normal control samples, (H) *Ki-67* in gastric tumor samples with clinical stage and TNM, (I) *Ki-67* in gastric tumor samples with different anatomical sites of gastric region and gastric tumor samples with different survival status.

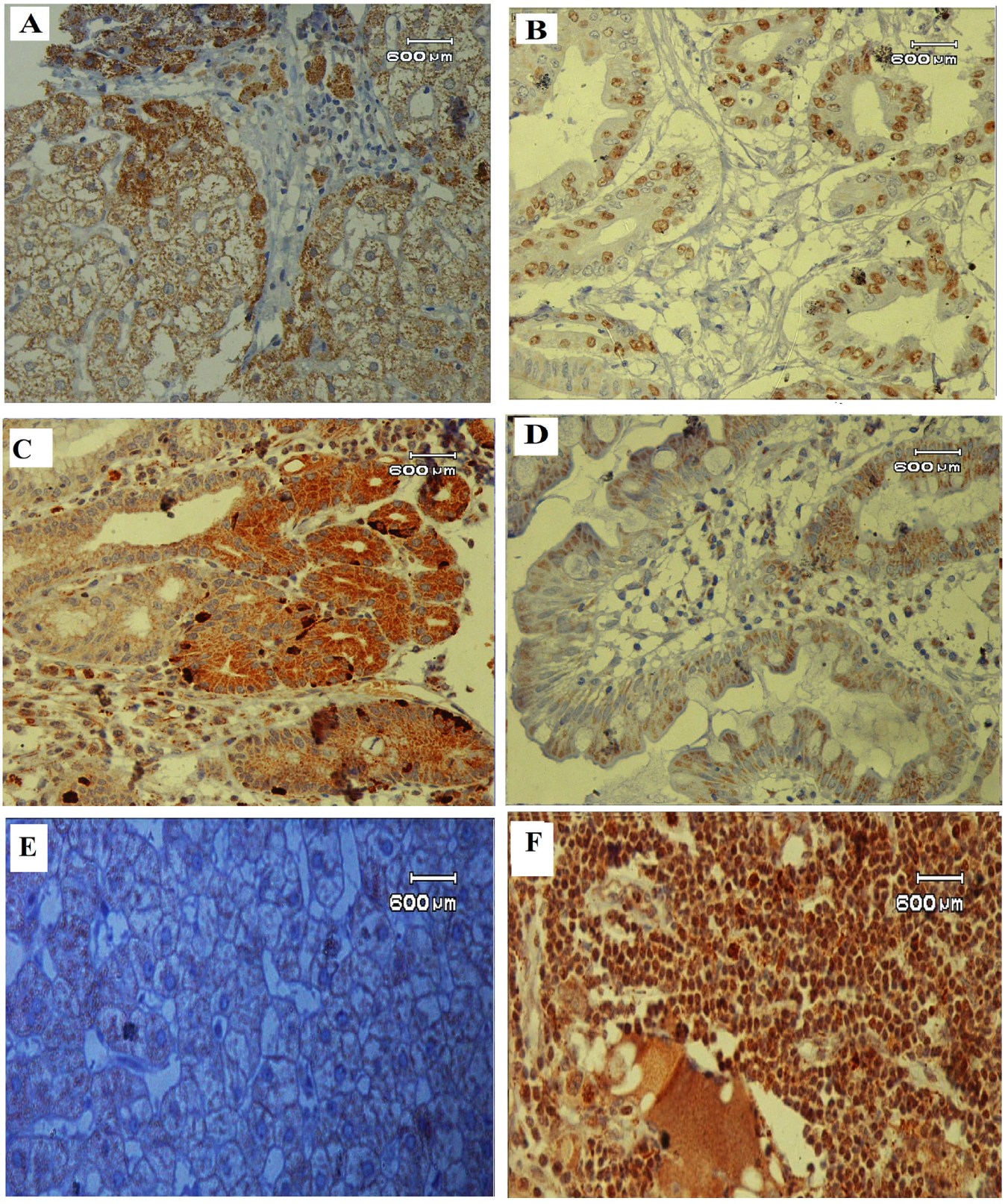
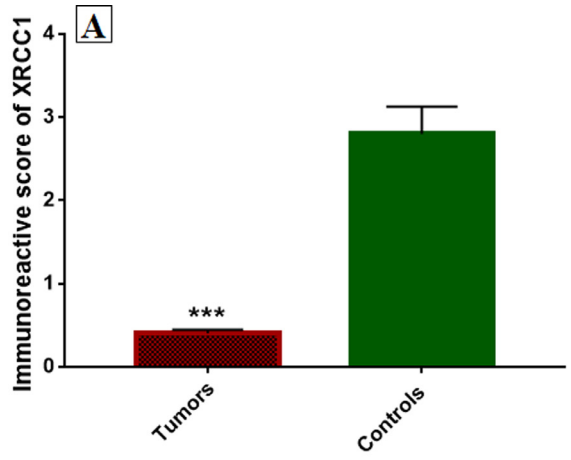
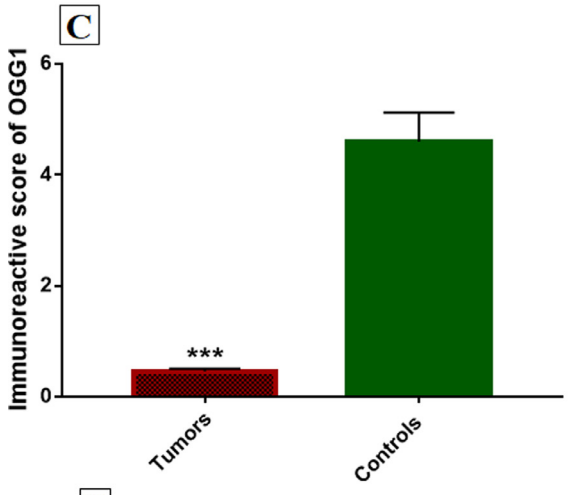



**Fig. 2** Immunohistochemistry analysis in gastric cancer. Immunohistopathological analysis was performed to examine the *XRCC1* expression on (A) uninvolved healthy gastric tissue control (B) gastric cancer tissue. Immunohistopathological analysis was performed to examine the *OGG1* expression on (C) uninvolved healthy gastric tissue control (D) gastric cancer tissue. Immunohistopathological analysis was performed to examine the *Ki-67* expression on (E) uninvolved healthy gastric tissue control (F) gastric cancer tissue. Scale bar 600 µm, magnification 20 ×.



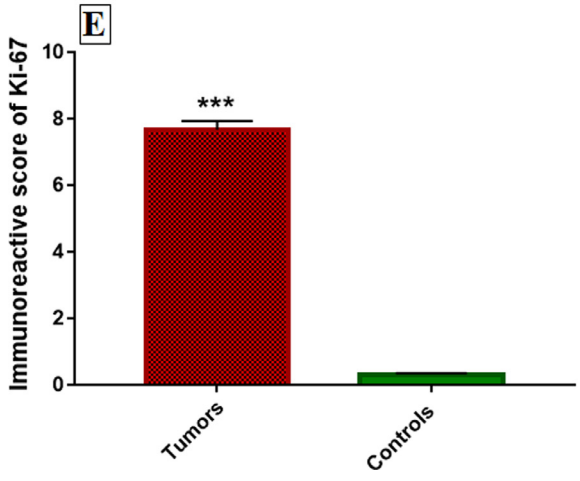

**B** **Immunoreative Intensity of XRCC1 in gastric carcinoma**

| Parameter | | Weak % | Moderate % | Strong % | p-value |
|---|---|---|---|---|---|
| **XRCC1** | | | | | |
| **C-stage** | I-II | 61% | 28% | 11% | |
| | III-IV | 71% | 17% | 12% | 0.31 |
| **T-stage** | T1-T2 | 57% | 29% | 14% | |
| | T3-T4 | 63% | 21% | 16% | 0.07 |
| **N-stage** | N0 | 78% | 14% | 08% | |
| | N1-N2 | 82% | 12% | 06% | 0.0001 |
| **M-stage** | M0 | 76% | 15% | 08% | |
| | M1 | 72% | 17% | 11% | 0.33 |
| **Types** | Adeno-carcinoma | 69% | 23% | 07% | |
| | Signet Ring cell carcinoma | 50% | 30% | 20% | 0.36 |
| | GIST | 50% | 38% | 12% | |
| | Neuroedndocrine Tumor | 75% | 25% | 0 | |




**D** **Immunoreative Intensity of OGG1 in gastric carcinoma**

| Parameter | | Weak % | Moderate % | Strong % | p-value |
|---|---|---|---|---|---|
| **OGG1** | | | | | |
| **C-stage** | I-II | 53% | 37% | 10%) | 0.38 |
| | III-IV | 67% | 21% | 12% | |
| **T-stage** | T1-T2 | 61% | 28% | 11% | |
| | T3-T4 | 68% | 26% | 06% | 0.04 |
| **N-stage** | N0 | 70% | 22% | 08% | 0.0009 |
| | N1-N2 | 78% | 18% | 06% | |
| **M-stage** | M0 | 77% | 17% | 06% | 0.37 |
| | M1 | 61% | 28% | 11% | |
| **Types** | Adeno-carcinoma | 77% | 15% | 08% | 0.2 |
| | Signet Ring cell carcinoma | 60% | 30% | 10% | |
| | GIST | 88% | 12% | 0 | |
| | Neuroedndocrine Tumor | 75% | 12.5% | 12.5% | |




**F** **Immunoreative Intensity of Ki-67 in gastric carcinoma**

| Parameter | | Weak % | Moderate % | Strong % | p-value |
|---|---|---|---|---|---|
| **Ki 67** | | | | | |
| **C-stage** | I-II | 13% | 18% | 69% | |
| | III-IV | 16% | 38% | 46% | 0.42 |
| **T-stage** | T1-T2 | 11% | 19% | 69% | |
| | T3-T4 | 21% | 29% | 50% | 0.17 |
| **N-stage** | N0 | 14% | 38% | 48% | |
| | N1-N2 | 12% | 22% | 66% | 0.04 |
| **M-stage** | M0 | 13% | 17% | 70% | |
| | M1 | 17% | 33% | 50% | 0.46 |
| **Types** | Adeno-carcinoma | 09% | 24% | 67% | |
| | Signet Ring cell carcinoma | 20% | 30% | 50% | 0.4 |
| | GIST | 2.5% | 12.5% | 75% | |
| | Neuroedndocrine Tumor | 12% | 25% | 63% | |

**Fig. 3** Immunoreactive score of *XRCC1*(A), *OGG1* (C) and *Ki-67* (E) in gastric tissue samples. Immunoreactive intensity of *XRCC1*(B), *OGG1* (D) and *Ki-67* (F) and its association with different histopathological parameters of gastric tissue samples.

($r=0.778^{**}$, $p<0.0001$), N-stage vs M-stage ($r=0.502^{**}$, $p<0.0001$), M-stage vs survival ($r=-0.301^{*}$, $p<0.03$) (Table 3).

In case of mRNA expression analysis, when gene-gene relationship was explored, we observed a positive Spearman correlation between *OGG1* vs *XRCC1* ($r=0.364^{*}$, $p<0.02$) and a negative correlation between *OGG1* vs *Ki-67* ($r=-0.462^{**}$, $p<0.001$) in GC tumors. (*indicate the level of significance of spearman correlations as calculated by SPSS). A negative correlation was also observed between *XRCC1* vs *Ki-67* ($r=-0.589^{**}$, $p<0.001$), in GC tumors as shown in Table 3.

For protein expression, we observed a significant positive Spearman correlation between *OGG1* vs *XRCC1* ($r=0.532^{**}$, $p<0.001$) and significant negative Spearmen correlation between *OGG1* vs *Ki-67* ($r=-0.382^{*}$, $p<0.02$)

**Table 3** Correlations between BER pathway genes (*OGG1, XRCC1*), proliferation marker (*Ki-67*) expression and clinco-pathological characteristics of Gastric cancer[†].

| | Clinical | T-stage | N-stage | M-stage | *OGG1* | *XRCC1* | *Ki-67* |
|---|---|---|---|---|---|---|---|
| **mRNA Level** | **Clinical** | **0.905**** | **0.778**** | **0.637**** | **−0.490**** | **−0.336*** | **0.379**** |
| | **T-stage** | | **0.520**** | **0.603**** | **−0.594**** | **−0.603**** | **0.543**** |
| | **N-stage** | | | **0.502**** | **−0.562***** | **−0.363*** | **0.462**** |
| | **M-stage** | | | | −0.056 | −0.254 | 0.083 |
| | ***OGG1*** | | | | | **0.364*** | **−0.462**** |
| | ***XRCC1*** | | | | | | **−0.589**** |
| | *Ki-67* | | | | | | |
| **Protein Level** | **Clinical** | **0.905**** | **0.778**** | **0.637**** | **−0.304*** | −0.121 | **0.289*** |
| | **T-stage** | | **0.520**** | **0.603**** | **−0.325*** | **−0.362*** | **0.349*** |
| | **N-stage** | | | **0.502**** | **−0.434**** | **−0.312*** | **0.309*** |
| | **M-stage** | | | | −0.139 | **−0.459**** | **0.366*** |
| | ***OGG1*** | | | | | **0.532**** | **−0.382*** |
| | ***XRCC1*** | | | | | | **−0.601***** |
| | **Ki-67** | | | | | | |

[†] Spearman Correlation Coefficients. The expression levels of *OGG1, XRCC1* and *Ki-67* for Patient Cohort were based on the relative mRNA level.

* $p < 0.05$. The p values were computed using one way ANOVA and $\chi$2-test.

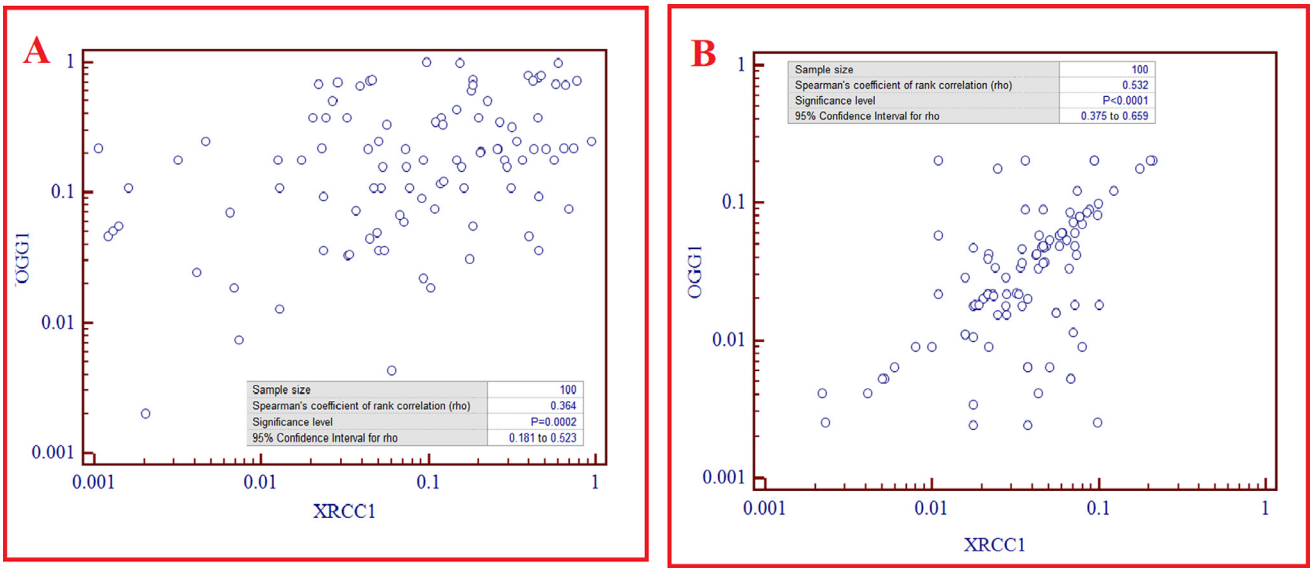



**Fig. 4** Co-expression analysis of *OGG1* and *XRCC1* at mRNA level (A) and protein level (B) in gastric tissue samples.

and *XRCC1* vs *Ki-67* ($r = -0.601$***, $p < 0.001$) in tumors (Table 3).

Co-expression of *XRCC1* and *OGG1* gene was also assessed at mRNA (Fig. 4A) and protein level (Fig. 4B) of patients. Co-expression analysis showed that *XRCC1* gene co-expressed with *OGG1* gene in GC tumors.

## Measurement of 8-hydroxy-2′-deoxyguanosine level in study cohort

The level of 8–hydroxy-2′-deoxyguanosine (8-OHdG) was measured and significantly higher levels were observed in GC tumors ($p < 0.0001$) when compared with controls, as shown in Fig. 5A. The level of 8-OHdG was further correlated with different histopathological parameters. Significant increased level of 8-OHdG was observed in advanced clinical-stages (III-IV, $p < 0.002$) and advanced T-stage (T3-T4; $p < 0.03$) compared to early clinical stages (I-II) and early T-stages (T1-T2), as shown in Fig. 5B. In case of N-stage of GC tumors, significantly increased 8-OHdG level was observed in advanced N-stages (N2-N3; $p < 0.001$) compared to early N-stages (N0-N1) (Fig. 5B).

## DNA fragmentation assay of BER pathway genes

DNA fragmentation assay was performed to measure the level of DNA damage in GC tumors and adjacent non-cancerous sections used as controls (Fig. 6A, 6B). Significantly higher frequency of fragmented DNA was observed in GC tumors ($p < 0.02$) compared to control tissues, as shown in Fig. 6C.

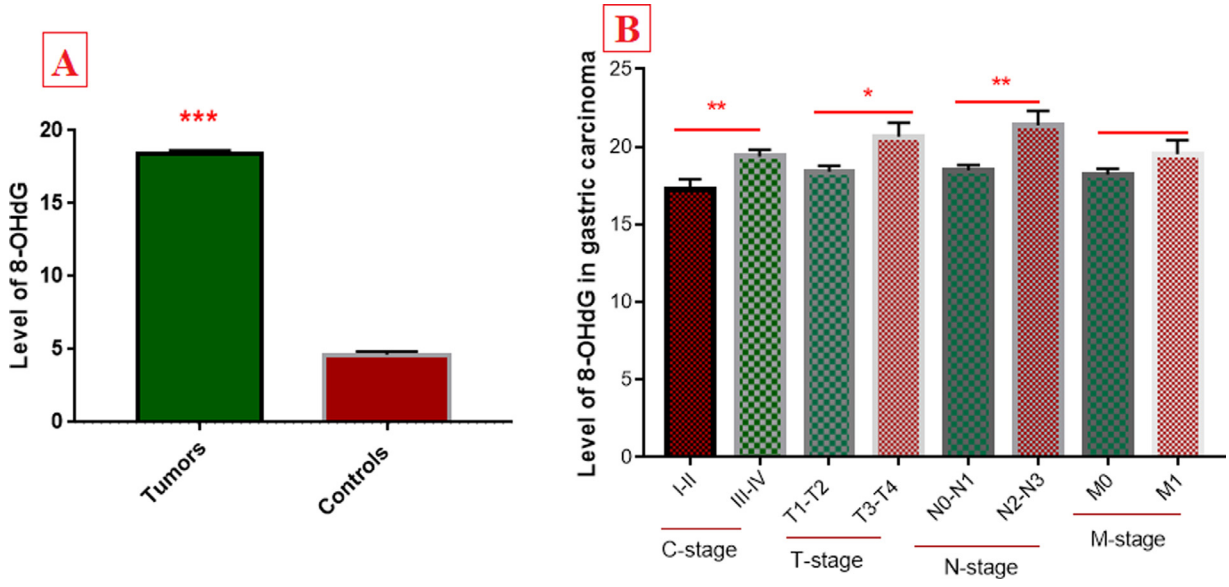



**Fig. 5** (A) Level of 8-OHdG was measured in gastric cancer tumors and adjacent controls. (B) Association of 8-OHdG level with different histopathological parameters of gastric cancer.

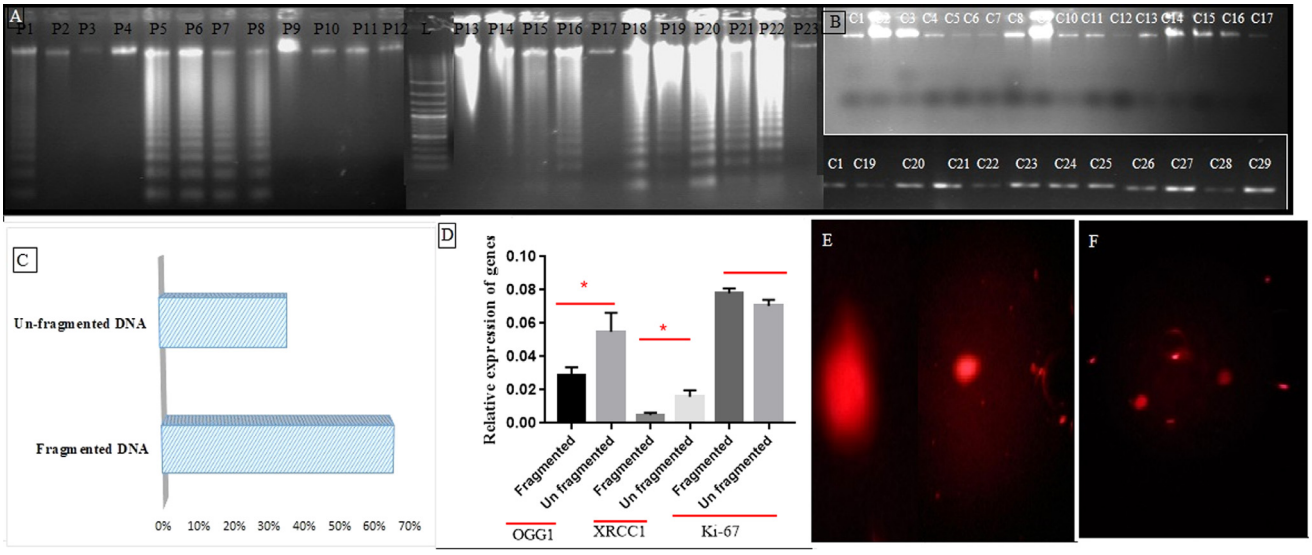



**Fig. 6** DNA fragmentation assay in (A) gastric tumor section (B) adjacent control section. (C) Percentage of fragmented and unfragmented DNA in gastric tumors. (D) Relative expression of selected BER patway genes in fragmented and unfragmented DNA of gastric tissue. Comet assay in (E) gastric tissue sample and (F) adjacent uninvolved tissue taken as controls.

Frequency of fragmented DNA was observed higher in advanced T-stages (T3-T4; $p < 0.03$), advanced N-stages (N1-N2; $p < 0.03$) and advanced M-stage (M1; $p < 0.02$) tumors when compared with early TNM stage of GC tumors. Further analysis showed that *XRCC1* ($p < 0.022$) and *OGG1* $p < 0.02$) downregulation was observed higher in tumor tissues with fragmented DNA when compared with tissues with un-fragmented DNA, as shown in Fig. 6D.

DNA damage was further confirmed by performing the comet assay in 100 gastric tissue samples and adjacent uninvolved sections used as controls. Number of cells with intact DNA and with comets were counted in gastric tumors tissues (Fig. 6E) and in adjacent control sections (Fig. 6F). Number of cells with comets were observed significantly higher in GC tumors compared to control sections ($p < 0.01$).

## ROC curve analysis

ROC curve analysis was performed to assess the diagnostic value of selected BER pathway genes such as *XRCC1* (Fig. 7A) and *OGG1* (Fig. 7B) in gastric cancer. After the generation of ROC curve, area under the curve (AUC) and 95%confidence interval (CI) were calculated. The area under the curve for *XRCC1* gene was 99.8 (95% CI: 0.976–1.00; $p < 0.0001$) and for *OGG1* gene was 93.7 (95% CI:0.890–

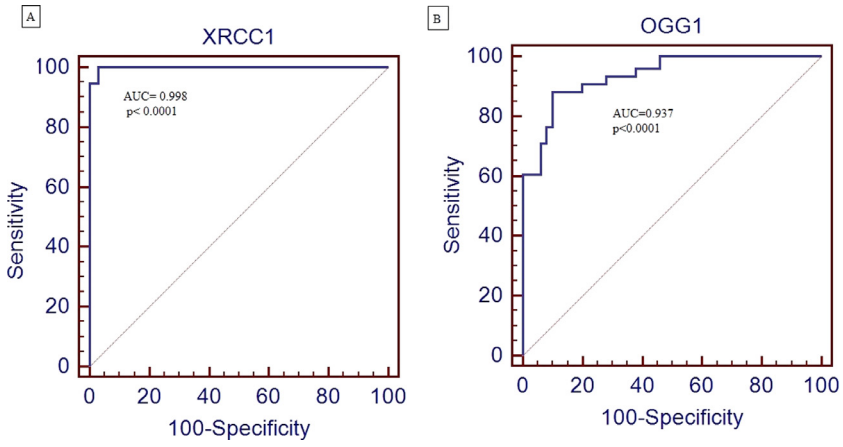



**Fig. 7** ROC curve analysis of *XRCC1* (A) and *OGG1* gene (B) in gastric cancer patients.

0.968; $p < 0.0001$). Results for ROC analysis are shown in Fig. 7.

## Discussion

The morphologic, biologic and genetic heterogeneity of gastric cancer makes it an appealing model for studying carcinogenesis. Various genetic, epigenetic and molecular alterations of tumor suppressor genes, oncogenes, regulators of cell cycle, cell signaling molecules and DNA repair genes are found in gastric cancer that underlie the malignant transformation of gastric mucosa during the multistep process of gastric carcinogenesis [19]. Nevertheless, most of the earlier studies have focused on mutation analysis of DNA repair genes [20,21] and very few studies on expression deregulation of BER pathway genes in gastric cancer [22,23]. In this study, we have combined the approaches for visualization and quantification of DNA repair gene expression variations, cell proliferation and DNA damage in order to learn about interrelationships of these phenomena in the context of gastric carcinogenesis.

Initially, we performed a systematic review to assess the role of two BER pathway genes in carcinogenesis. Only 4 studies (2 for each selected gene) out of 20 studies, were found on expression deregulation of said genes in gastric cancer with small study cohort [24–42]. In the second step, we analyzed the transcription and translation profile of *XRCC1* and *OGG1* genes. Our study revealed reduced expression of *XRCC1* and *OGG1* in GC tumors compared to control tissues. This downregulation was more pronounced in advanced TNM stages. This variation and contrariety between the expression patterns of controls and tumor tissues is suggestive of genetic heterogeneity in gene expression, which may be due to different genetic and epigenetic alterations or some other environmental factors.

Present study also examined the level of 8-OHdG in study cohort and increased 8-OHdG was observed in GC tumors compared to controls. The reduced expression of these DNA repair genes may likely exhibit an impaired or reduced DNA repair activity, consequently leading to the accumulation of more 8-OHdG in gastric cancer. The resultant accumulation of 8-OHdG subsequently induces endogenous oxidative stress to DNA, thereby facilitating tumor initiation and progression [43]. Other plausible explanation of downregulation of *XRCC1* and *OGG1* gene is the presence of mutant genotypes in cancer patients. It has earlier been reported that cancer patients with low *OGG1* level exhibit high number of mutant genotypes than patients with high *OGG1* level [44]. Our results are concurrent to various earlier studies where significant downregulation in relative expression of *XRCC1* and *OGG1* was observed in head and neck cancer [45,12] and cervical cancer [7].

To check the diagnostic value of both selected molecules *XRCC1* and *OGG1*, ROC curve analysis was performed. ROC curve for *XRCC1* and *OGG1* showed that the AUC value was nearly 99.8 and 93.7 respectively, indicating that the expression level of *XRCC1* and *OGG1* might be used to diagnosis of gastric cancer.

Studies have shown that deregulation in DNA repair gene usually result in the accumulation of DNA adducts which in turn may result in increased proliferation, loss of apoptotic function, and chromosomal instability [46–48]. To test this hypothesis expression profile of *Ki-67* was observed in gastric tumor samples along with adjacent non-cancerous controls tissues. Significant upregulation of expression was observed in GC tumors compared to control tissues. This overexpression of *Ki-67* provided evidence of multistep deregulation of proliferation process in gastric cancer patients. It has been observed in various studies that upregulation of *Ki-67* might be associated with growth arrest and deregulation of proliferative process [5,4]. Since uncontrolled proliferation is a common feature of tumor cells, therapeutic agents that target *Ki-67* may be useful tools in cancer treatment. For gene-gene interaction, significant upregulation of *Ki-67* was correlated with deregulation of DNA repair pathway genes leading to excessive proliferation and more aggressive tumorigenesis in GC patients. Similar results have already been reported in head and neck cancer [5] and thyroid cancer [49].

Genomic instability is a characteristic of most human malignancies and it is considered a hallmark of cancer cells. Genomic instability is caused by downregulation of DNA damage response pathway and DNA repair pathway in carcinogenesis. To support this idea, genomic instability was measured in GC tumors using the fragmentation assay followed by comet assay and significant higher number of fragmented DNA and

comets were observed in GC tumors compared to adjacent uninvolved control tissues.

Analysis of association between genomic instability and expression profiling of these genes showed that more downregulated levels were observed in GC tumors with fragmented DNA compared to those with un-fragmented DNA. This showed that DNA repair gene deregulation results in accumulation of cytotoxic and mutagenic base lesions. Downregulation in DNA repair genes also results in hypersensitivity to alkylation-induced DNA damage [50,51] and improper single strand break repairs due to inefficient DNA termini clean up and nick ligation [52] which leads to increased proliferation and carcinogenesis.

This study revealed downregulation of *XRCC1* and *OGG1* expression which signifies the causal involvement of BER pathway in gastric cancer initiation and progression. Our findings suggest that compromised and aberrant BER pathway genes such as *XRCC1* and *OGG1* are an indicator of DNA damage and may be considered a possible biomarker for improved diagnosis and prognosis of gastric cancer. Based on this study it can be concluded that inactivation of DNA repair genes may be seen as an important event in tumorigenesis. This inactivation results in reduced genomic stability which may lead to genetic aberrations at another important gene loci. This may enable a cell, with reduced repair capacity, to undergo uncontrolled proliferation, instead of cell death.

## Conflict of interest

The authors declare that they have no conflict of interests.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.06.002.

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