RESEARCH ARTICLE

XRCC1 Gene Polymorphism, Clinicopathological Characteristics and Stomach Cancer Survival in Thailand

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Abstract

Background: Stomach cancer is one of leading causes of death worldwide. In Thailand, the incidence and mortality of stomach cancer are in the top ten for cancers. Effects of DNA repair gene X-ray repair cross complementary protein 1 (XRCC1) polymorphisms and clinicopathological characteristics on survival of stomach cancer in Thailand have not been previously reported. The aim of this study was to investigate the effects of XRCC1 gene and clinicopathological characteristics on survival of stomach cancer patients in Thailand. Materials and Methods: Data and blood samples were collected from 101 newly diagnosed stomach cancer cases pathologically confirmed and recruited during 2002 to 2006 and followed-up for vital status until 31 October 2012. Genotype analysis was performed using real-time PCR-HRM. The data were analyzed using the Kaplan-Meier method to yield cumulative survival curve, log-rank test to assess statistical difference of survival and Cox proportional hazard models to estimate adjusted hazard ratio. Results: The total followed-up times were 2,070 person-months, and the mortality rate was 4.3 per 100 person-months. The median survival time after diagnosis was 8.07 months. The cumulative 1-, 3-, 5-years survival rates were 40.4%, 15.2% and 10.1% respectively. After adjustment, tumor stage was associated with an increased risk of death (p=0.036). The XRCC1 Gln339Arg, Arg/Arg homozygote was also associated with increased risk but statistically this was non-significant. Conclusions: In addition to tumor stage, which is an important prognostic factor affecting to the survival of stomach cancer patients, the genetic variant Gln339Arg in XRCC1 may non-significantly contribute to risk of stomach cancer death among Thai people. Larger studies with different populations are need to verify ours findings. Keywords: XRCC1 polymorphism - survival - clinicopathological - stomach cancer - Thailand

Introduction

More than 990,000 cases of stomach cancer have been diagnosed and more than 738,000 deaths have occurred worldwide. The highest mortality rates were reported in Eastern Asia with a rate of 28.1 per 100,000 in males and 13.0 per 100,000 in females. The lowest mortality rates were reported in the Northern America (Ferlay et al., 2010). In Thailand, stomach cancer is one of the most common forms of malignancies. The overall estimated age-standardized incidence rate (ASR) for males was 4.5 per 100,000 for and 1.4 per 100,000 for females (Suwanrungruang et al., 2006).

The X-ray repair cross-complementing group 1 (XRCC1) is one type of genetic variant that has been implicated in cancer susceptibility. From the evidence 297 case-control studies found XRCC1 Arg399Gln increases risk for overall cancer (Yi et al., 2013) and many studies suggest that the XRCC1 gene is one of the most important genetic risk factors for stomach cancer (Hong et al., 2009; Engin et al., 2011; Yuan et al., 2011; Chen et., 2012; Pan et al., 2012; Qiao et al., 2013) and previous studies have pointed to XRCC1 polymorphism as an important prognostic factor for survival of gastric cancer (Shim et al., 2010; Tahara et al., 2011; Deng et al., 2014; Zhang et al., 2014).

In terms of clinicopathological characteristics, Previous studies have reported tumor site, tumor size, lymph node ratio, staging of diseases, lymph node metastasis, tumor invasion, distant of metastases, Borrmann type, depth of invasion and surgical margin status all related to survival of stomach cancer patients (Yin
Materials and Methods

Study subjects
In total, 101 newly diagnosis stomach cancer patients were included in this study. All cases were histologically confirmed and diagnosed according to the International Classification of Diseases for Oncology (ICD-O 3rd).

Subjects were recruited from Srinagarind Hospital and Khon Kaen Regional Hospital, Khon Kaen Province, Northeast Thailand, during 2002 to 2006. All of patients were followed-up until death or the end of the study (31 October, 2012). Factors of interest were retrieved from medical records including age at diagnosis, gender, site of diseases, surgery type, histological type, histological grading and stage of disease. \( XRCC1 \) genotyping was performed (described below). The classical endpoint in this study is survival time of stomach cancer.

Laboratory methods

Specimen collection and DNA extraction: Blood samples were taken from all stomach cancer patients diagnosis in the study period (n=101). Whole blood samples of 3-5 ml were collected and centrifuged at 3,000 rpm for 15 minute to separate plasma, buffy coat and red blood cells. All specimens were stored at -20° C at the cancer unit, Faculty of Medicine, Khon Kaen University. The genomic DNA was extracted from the buffy coat at Nagoya city university medical school, Japan.

PCR amplification and genetic polymorphisms detection

The DNA analyses were performed by using real-time polymerase chain reaction with high resolution melting technique (Real-time PCR-HRM). DNA amplification was performed in a 96-well plate in the light Cycler® 480 Real-Time PCR System. The amplification of \( XRCC1 \) Gln399Arg gene was used two primers, [Forward]: 5'-AGT GGG TGC TGG ACT GTC-3' and [Reverse]: 5'-TTG CCC AGC ACA GGA TAA-3'. The HRM data were analyzed using the light Cycler® 480 Gene Scanning software version 1.5(Roche) and was performed at Department of Microbiology, Faculty of Medicine, Khon Kaen University.

Statistical analysis

Survival times of patient were calculated for each patient and were started from the date of diagnosis until the date of death or the end of follow-up (31 October, 2012). Percentages were used to describe categorical data and means with standard deviations or medians with ranges were used to describe continuous data. The observed survival rate was calculated and summarized using Kaplan-meier survival curves. The statistics used to compare survival between groups was performed by using the log-rank test. The univariate and multivariate Cox proportional hazard regression models were used to estimate the association between explanatory variable and survival experience, presented crude hazard ratios (HR) and adjusted HRs and their 95% confidence interval (CI). All analysis was conducted using the SAS statistical package (version 9.3; SAS institute, Cary, NC) and significance level of 0.05 was used for all analysis.

The Ethics Consideration

The study was approved by the Khon Kaen University Ethics Committee for Human Research. The reference number is HE561259.

Results

Demographic characteristics of stomach cancer

The results of the descriptive analysis were summarized in Table 1. Of the 101 patients with stomach cancer, 57 (56.4%) were males. The mean age was 52.7 years. Most of the cancer patients were married 78.2 %, had only a primary school education were 74.3 % and farmers or agricultural worker were 69.3 %. Table 2 shows the frequencies and the contribution of pathological characteristics of cases. The most commonly specified anatomical sites of stomach cancer were the antrum (45.6 % of all cases) and the cardia (16.8 % of all cases). The most common type of surgery was subtotal gastrectomy (49.5 %). Regarding histopathology, the most frequently specified histological type of malignancy was signet ring cells carcinoma (24.7 % of all cases), and in most patients histological grade was assessed as poorly differentiated (58.4 %) or unable to be assessed (28.4 %). Stage IV cancers (53.5 %) preponderated the majority of the patients. The allele frequencies of \( XRCC1 \) Gln399Arg polymorphisms for Gln/Gln, Gln/Arg and Arg/Arg genotypes were 47.5 %, 40.6 % and 11.9 %, respectively.

Survival rate of stomach cancer

The total follow-up person time was 2,070 person-months, and the overall mortality rate was 4.3 per 100 person-months (95%CI: 3.49 to 5.35). Table 3 presents the survival rates. The cumulative 3-, 6- and 9 months, 1-, 3- and 5-years survival rates were 86.9 %, 63.7 %, 46.5 %, 40.4 %, 15.2 % and 10.1 %, respectively. The median survival time of stomach cancer after diagnosis was 8.07 months (95%CI: 6.00 to 10.23; Figure 1). The Figures 2-5 presented survival times of stage of diseases, histology type, histology grading and \( XRCC1 \) polymorphisms. The median survival time of Stage I, Stage II, Stage III, Stage IIB, Stage IV and Unknown stage were 9.10, 35.40, 22.90, 14.14, 8.67, 6.27 months respectively. Regarding to \( XRCC1 \) polymorphisms, the median survival time of Arg/Arg, Gln/Arg and Gln/Gln genotype were 15.60, 12.30 and 7.33 months respectively.
The associated of Clinicopathological and XRCC1 gene Polymorphisms with survival of stomach cancer

Table 4 shows after adjusting for lymph node metastasis, comorbidity and complication. Tumour stage IV and Unknown stage lead to increased risked of death (HR: 3.6; 95%CI: 1.35 to 9.43; HR: 3.0; 95%CI: 1.08)

Table 3. Survival Rate of Stomach Cancer After Diagnosis

| Survival time (Months) | Median Survival time | 95% CI | Survival rate (%) | 95% CI |
|------------------------|----------------------|--------|-------------------|--------|
| 3 Months               | 1.9                  | 0.63-2.07 | 86.9              | 78.49-92.17 |
| 6 Months               | 3.5                  | 2.80-4.60 | 63.7              | 53.36-72.27 |
| 9 months               | 5.1                  | 4.00-5.70 | 46.5              | 36.43-55.90 |
| 1 Year                 | 5.5                  | 4.40-6.07 | 40.4              | 30.73-49.87 |
| 3 Years                | 6.9                  | 5.70-8.73 | 15.2              | 8.93-22.90 |
| 5 Years                | 7.8                  | 5.80-10.23 | 10.1              | 5.17-16.97 |

Figure 1. Overall Survival Curve of Stomach Cancer

Figure 2. Survival Curve of Stomach Cancer by Stage of Diseases

Figure 3. Survival Curve of Stomach Cancer by Histology Type

Figure 4. Survival Curve of Stomach Cancer by Histology Type
Table 4. Pathological and XRCC1 Gene as Effected to Survival of Stomach Cancer (Multivariate Analysis)

| Variable                     | Number(%) | Median time (Months) | Crude HR (95% CI) | Adjusted HR (95% CI) | p-value |
|------------------------------|-----------|---------------------|-------------------|----------------------|---------|
| Gender                       |           |                     |                   |                      |         |
| Male                         | 57(56.4)  | 10.3( 6.1-14.3)     | 1                 | 1                    | 0.306   |
| Female                       | 44(43.6)  | 8.1(5.7-12.3)       | 1.2 (0.81-1.87)   | 1.2 (0.81-1.89)      |         |
| Age                          |           |                     |                   |                      |         |
| < 60                         | 70(69.3)  | 8.6(6.7-12.3)       | 1                 | 1                    | 0.414   |
| > 60                         | 31(30.7)  | 10.1(4.8-17.4)      | 0.9(0.62-1.49)    | 1.0 (0.59-1.44)      |         |
| Site of diseases             |           |                     |                   |                      | 0.308   |
| Fundus, Pylorus, Body        | 10(10.4)  | 13.7(5.1-20.2)      | 1                 | 1                    |         |
| Cardia                       | 17(16.8)  | 12.8(3.4-20.2)      | 2.2 (0.87-5.65)   | 2.3 (0.90-6.08)      |         |
| Antrum                       | 46(45.6)  | 7.8(5.6-11.9)       | 2.0 (0.86-4.81)   | 2.1 (0.85-5.04)      |         |
| Stomach, NOS                 | 28(27.2)  | 8.4(5.5-12.3)       | 2.1 (0.89-5.31)   | 2.4 (0.95-6.02)      |         |
| Type of surgery              |           |                     |                   |                      |         |
| Gastric mucosa biopsy        | 21(21.0)  | 6.5(2.3-12.3)       | 1                 | 1                    | 0.149   |
| Subtotal gastrectomy         | 50(49.5)  | 11.6(6.1-17.3)      | 0.4 (0.05-2.65)   | 0.3 (0.04-3.05)      |         |
| Near total gastrectomy       | 7(6.9)    | 11.9(2.8-64.6)      | 0.3 (0.03-2.47)   | 0.2 (0.02-2.10)      |         |
| Total gastrectomy            | 15(14.5)  | 11.5(1.9-31.4)      | 0.3 (0.04-2.45)   | 0.3 (0.02-2.73)      |         |
| Other                        | 8(8.1)    | 4.5(1.2-7.8)        | 1.2 (0.14-9.45)   | 1.3 (0.15-11.85)     |         |
| Histology type               |           |                     |                   |                      | 0.657   |
| Tubular adenocarcinoma, Diffuse type | 6(6.0) | 6.7(3.5-NA)         | 1                 | 1                    |         |
| Signet ring cell carcinoma   | 25(24.7)  | 10.2(5.8-20.6)      | 0.6 (0.08-4.60)   | 0.7 (0.08-5.54)      |         |
| Adenocarcinoma, NOS          | 69(69.3)  | 8.7(5.7-12.9)       | 0.7 (0.09-4.72)   | 0.9 (0.11-6.81)      |         |
| Histology grading            |           |                     |                   |                      | 0.638   |
| Well differentiated           | 10(9.9)   | 6.8(2.3-31.4)       | 1                 | 1                    |         |
| Moderately differentiated    | 11(10.9)  | 12.8(5.7-21.3)      | 0.8 (0.45-1.63)   | 0.7 (0.27-1.88)      |         |
| Poorly differentiated         | 59(58.4)  | 8.7(6.7-13.0)       | 1.3 (0.77-1.79)   | 0.8 (0.37-1.70)      |         |
| Grade can't be assessed       | 21(21.8)  | 6.1(3.5-14.8)       | 0.8 (0.46-1.37)   | 0.6 (0.25-1.54)      |         |
| Stage of diseases            |           |                     |                   |                      | 0.036   |
| Stage IB+II                  | 8(7.9)    | 39.1(3.4-NA)        | 1                 | 1                    |         |
| Stage IIIA+IIIB              | 15(14.8)  | 15.6(5.4-35.1)      | 1.4 (0.49-4.15)   | 1.9 (0.63-5.71)      |         |
| Stage IV                     | 54(53.5)  | 8.7(5.8-11.5)       | 2.8 (1.09-7.02)   | 3.6 (1.35-9.43)      |         |
| Unknown Stage                | 24(23.8)  | 6.2(4.0-12.3)       | 2.3 (1.87 - 6.21) | 3.0 (1.08-8.34)      |         |
| XRCC1 G339A genotype         |           |                     |                   |                      | 0.136   |
| Gln/Gln                      | 48(47.5)  | 7.3(5.5-8.7)        | 1                 | 1                    |         |
| Gln/Arg                      | 41(40.6)  | 12.3(6.5-17.5)      | 0.6 (0.40-0.98)   | 1.0 (0.52-2.02)      |         |
| Arg/Arg                      | 12(11.9)  | 15.6(3.3-39.1)      | 1.6 (1.02-2.50)   | 1.8 (0.89-3.45)      |         |

*Stomach cancer; 95% CI, 95% confidence interval, were adjusted for complication, comorbidity and metastasis using Cox proportional hazard regression models, p-value from Partial likelihood ratio test; NA, Not Applicable; NOS, not otherwise specified, Other = Gastrojejunectomy, Hemigastrectomy and Esophagogastrectomy

Table 5. Final Multivariate Model of Significant Factors Independently Associated with Hazard of Death

| Variable        | HRa     | 95% CI      | p-value |
|-----------------|---------|-------------|---------|
| Stage IV        | 1.7     | 1.09-2.59   | 0.019   |

*Stomach cancer; HRa, Adjusted Hazard Ratio; 95% CI, 95% confidence interval p-value base on stepwise cox proportional hazards regression to 8.34). The Polymorphisms of XRCC1 Gln339Arg were associated increased risked of death with Arg/Arg homozygote but we can’t demonstrated statistically significant. Table 5 show the final multivariate model of significant factors independently associated with hazard of death base on stepwise Cox proportional hazards regression and found a tumour stage IV was associated with hazard of death 1.7-fold (95%CI: 1.09 to 2.59).

Discussion

Our study investigated the factors associated with mortality among stomach cancer patients. This is firstly reported on the effected of the XRCC1 gene and clinicopathological characteristics on the survival of stomach cancer patients among Thai peoples. Our resulted found the stage of diseases was the factors affected to survival of patients, which is consistent with previous studies have been reported. They found out that the staging of diseases were impotent factors affected to survival of gastric cancer patients especially advance stage of diseases (Choi et al., 2011; Kwon et al., 2014).
The study on the effect of the XRCC1 gene to survival of stomach cancer patients, many studies have been explored much on the associated of XRCC1 gene and clinical outcome to survival of patients under treatment by chemotherapy (Liu et al., 2007; Wang et al., 2012; Zou and Yang, 2012; Xu et al., 2014) but non studies have been conducted in Thailand. Our results have found out that the XRCC1 Gln399Arg, Arg/Arg homozygote was affected to survival of stomach cancer patients but statistically non significant.

The tumor location and type of surgeries has important factors affected to survival of stomach cancer patients. Ours study found the tumor location and type of surgeries were not increases risked of death. This is inconsistent with previously studies done in Korea, France and China they found out that the location of cancer in the stomach and type of surgeries were important factor that effected to survival of stomach cancer patients (Choi et al., 2011; Deng et al., 2014; Son et al., 2014; Herbreteau et al., 2015). The histology grading and histology type, our study found not increases risked of death. Similar findings have been previously reported elsewhere (Kwon et al., 2014; Son et al., 2014).

In conclusion, our study suggests the stage of diseases is the factors affecting to survival of stomach cancer in Thai population. We did not find any effects of XRCC1 polymorphisms, tumor location, surgeries type, histology grading and histology type were associated with an increase risk of death of stomach cancer patients. It would be necessary to confirm these findings in the larger sample size.

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References

Chen B, Zhou Y, Yang P, et al (2012). Polymorphisms of XRCC1 and gastric cancer susceptibility: A meta-analysis. *Molecular Biology Reports*, 39, 1305-13.

Choi JY, Shim KN, Roh SH, et al (2011). [Clinicopathological characteristics of gastric cancer and survival improvement by surgical treatment in the elderly]. *The Korean journal of gastroenterology = Taejun Sohwagi Hakhoe chi*, 58, 9-19.

Deng J, Zhang R, Pan Y, et al (2015). Tumor size as a recommendable variable for accuracy of the prognostic prediction of gastric cancer: a retrospective analysis of 1,521 patients. *Annals of Surgical Oncology*, 22, 565-72.

Deng J, Zhang R, Pan Y, et al (2014). N stages of the seventh edition of TNM Classification are the most intensive variables for predictions of the overall survival of gastric cancer patients who underwent limited lymphadenectomy.
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the XRCC1 Arg399Gln polymorphism and risk of cancer: evidence from 297 case-control studies. *PloS One*, 8, 78071.

Yin C, Li D, Sun Z, et al (2012). Clinicopathologic features and prognosis analysis of mucinous gastric carcinoma. *Medical Oncology*, 29, 864-70.

Yuan T, Deng S, Chen M, et al (2011). Association of DNA repair gene XRCC1 and XPD polymorphisms with genetic susceptibility to gastric cancer in a Chinese population. *Cancer Epidemiology*, 35, 170-4.

Zhang X, Jiang LP, Yin Y, Wang YD. (2014). XRCC1 and XPD genetic polymorphisms and clinical outcomes of gastric cancer patients treated with oxaliplatin-based chemotherapy: a meta-analysis. *Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine*, 35, 5637-45.

Zou HZ, Yang SJ (2012). Prediction role of seven SNPs of DNA repair genes for survival of gastric cancer patients receiving chemotherapy. *Asian Pac J Cancer Prev*, 13, 6187-90.