7

Epidemiology and Polymorphisms Related to Bladder Cancer in Ecuadorian Individuals

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1. Introduction

Bladder cancer (BC) is the fourth most common cancer in men and the eighth most common in women being the responsible for annual deaths of 150,000 and is the seventh most prevalent type of cancer worldwide (Parkin, et al., 2005; Jemal, et al., 2009; Altayli, et al., 2009; Covolo, et al., 2008; Marmot, et al., 2007). In Ecuador the incidence rates of BC are 5.4% in males and 1.6% in females taking into account all cases of cancer diagnosed (Cueva & Yepez, 2009). In Argentina, it was reported as the fourth and the fourteenth most commonly diagnosed malignancy in men and women, respectively, with age-standardized incidence rate per 100,000 people around 15.1 (men) and 2.6 (women) in the period 1998 - 2002 (Pou, et al., 2011). The estimated downward trend in bladder cancer mortality over the last decades has been previously reported in countries of the European Union (Bosetti, et al., 2008) as well as South and North America (Bosetti, et al., 2005).

Susceptibility to BC is considered to depend on interaction between genetic factors and environmental chemical carcinogens. Bladder cancer involves a heterogeneous cell population, and numerous factors are likely to be involved in tumorigenesis (Hirao, et al., 2009). These factors result in uncontrolled growth of the cell population, decreased cell death, invasion and metastasis, and may influence the patient´s prognosis. Identification of the aggressive features of the cancer in patients with BC is very important for adequate management of this disease (Ha, et al., 2011).

Many studies have investigated the effects of gene polymorphism on the risk of cancer in humans (Paz-y-Miño, at al., 2010; Wacholder, et al., 2004; Marchini, et al., 2004). Single nucleotide polymorphisms (SNPs) are the most common type of gene polymorphism. Several millions of SNP variants have been identified. The risk of cancer associated with this type of polymorphism probably is not high, and the proportion of malignant tumors associated with a distinct polymorphism depends on the frequency of occurrence of this variant in the human population (Zaridze, 2008). Genetic polymorphisms that alter the activity of enzymes of biotransformation pathways have been reported to be associated with cancer development and progression (Franekova, et al., 2008).

In the other hand, molecular epidemiology of cancer studies, molecular markers of distribution of malignant tumors in the populations and their effects on individual are important to understand the risk of developing a disease. For an epidemiological study is

very important not only the source of the biological material, but also the individual information, that could be the factors influencing the risk of developing cancer. Among these can mention lifestyle factors as smoking, alcohol consumption, nutrition/diet, physical activity, environmental factors as occupation and exposure to carcinogens at workplace, familial and individual medical history, and many other variables (Zaridze, 2008). Many epidemiological studies have been conducted to investigate the putative association between polymorphic genes for biometabolism, environmental carcinogens, and the development of urinary tract cancer (Souto Grando, et al., 2009).

The association between cigarette smoking and cancer of the urinary tract has been extensively investigated in epidemilogy (Zeegers, et al., 2000). Cigarette smoking is the main bladder cancer risk factor for both men (60%) and women (25%) (Paz-y-Miño, et al., 2010); approximately half of male urinary tract cancers and one third of female urinary tract cancers may be attributable to cigarette smoking (Hecht, 2003). Over 60 carcinogens have been identified in cigarette smoke. Among these are polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene and aromatic amines, such as 2-naphtylamine and 4-aminobiphenyl, the organic benzene derivatives found in cigarettes and the reactive oxygen species (ROS) such molecular oxygen, hydrogen peroxide, and hydroxyl radicals (Ichimura, et al., 2004) increase the risk of developing this neoplasm by 25% (Paz-y-Miño, et al., 2010; Hecht, 2003; Luch, 2005). Molecular markers can be detected in tissues and biological liquids and characterize individual exposure to carcinogens, biological effect of the exposure, genetic susceptibility to the development of disease, and final result of carcinogenesis, i.e. tumor (Zaridze, 2008).

Many studies have indicated the relationship between different genetic polymorphisms and bladder cancer among the may appoint enzymes that perform a detoxifying function deactivate compounds and anions that are dangerous for the cell (Paz-y-Miño, et al., 2010). Cells are protected against metabolic ROS by several enzymatic and non-enzymatic defense systems, including superoxide dismutase (SOD), glutathione peroxidase (GPX) and reduced glutathione (Heistad, 2003). Three isoforms of SOD are present: Cu,Zn-SOD (SOD1 gene, cytosolic protein), Mn-SOD (SOD2 gene, mitochondrial protein) and EC-SOD (SOD3 gene, extracellular SOD) (Faraci & Didion, 2004). Manganese superoxide dismutase (MnSOD) has been the subject of particular interest as it is located in mitochondria and can be induced by several cytokines and by superoxide anion; it also appears to be involved in other processes, including tumor suppression and cellular differentiation (Charniot, et al., 2011).

In regards to GPX1, this is a major intracellular enzyme that catalyzes the degradation of peroxides by oxidizing glutathione with the formation of its conjugates, thereby preventing cellular injury (Deng, et al., 2008; Trošt, et al., 2010). Mutation in gene GPX1, which locates at chromosome 3p21, is one of the major factors regulating GPX1 activity. And among these, a genetic polymorphism at codon 198, resulting in either a proline (Pro) or leucine (Leu) at the corresponding position of the encoded peptide, have drawn increasing attention in the etiology of several cancers (Raaschou-Nielsen, et al., 2007; Ezzikouri, et al., 2010). In humans, the selenium-dependent activation of GPX 198Leu mutant enzyme is lower than for the GPX 198Pro wild-type enzyme (Hu, et al., 2010). And associations between low level of GPX1 activity in the circulation and increased risk of cancer were found in several cancer types including breast cancer (Arsova-Sarafinovska, et al., 2009; Hansen, et al., 2009); it is presumed that GPX1 Pro198Leu (C[T) polymorphism affecting GPX1 activity may be important for cancer development (Hu, et al., 2010).

The glutathione *S*-transferases (GSTs) are conjugation enzymes, which detoxify reactive chemical species, for example polycyclic aromatic hydrocarbons. Moreover these enzymes belong to a group of dimeric isozymes with various catalytic activities, which predominantly conjugate with electrophiles of glutathione conjugation and exert other noncatalytic functions. This isozyme is expressed in many tissues, including urinary bladder, and frequently overexpressed in carcinomas. The respiratory, urinary, and digestive tract epithelia express high levels of GSTP1 activity (Altayli, et al., 2009; Kopps, et al., 2008; Fishbain, et al., 2004)

There are five subclasses of the GST enzymes in humans: alpha, pi, mu, theta and zeta (Strange, et al., 2001). GSTM1, GSTT1, and GSTP1 are phase II enzymes (Rodriguez-Antona & Ingelman-Sundberg, 2006).

Altered substrate affinity has been shown in a polymorphism at exon 5 of the GSTP1 gene. Some studies have reported higher susceptibility to cancer in individuals carrying the variant GSTP1 allele, although contradictory results have also been obtained (Srivastava, et., 2005; Hu, et al., 1997).

A prevalent genetic polymorphism of the GSTP1 gene was reported differing only in a single A to G transition at nucleotide position 1578 corresponding to codon 105, resulting in an amino acid change from isoleucine to valine (Zimniak, et al., 1994; Harries, et al., 1997). The polymorphic forms were designated GSTP1a (Ile105, wild type) and b (Val105, mutant). Homozygosity for GSTP1 (Ile105Val) was found to be associated with a considerably higher risk for bladder cancer in patients in the United Kingdom (Harries, et al., 1997). In contrast, another study on Chinese benzidine workers diagnosed with bladder cancer indicated that GSTP1 Ile/Val and Val/Val polymorphism was a factor in disease occurrence (Ma, et al., 2003).

Association between oxidative stress and DNA damage has been well known and many studies have focused on the association between DNA damage and the development of certain diseases (Paz-y-Miño, et al., 2010; Padma, et al., 2011). DNA repair enzymes continuously monitor chromosomes to correct damaged nucleotide residues generated by exposure to cytotoxic compounds or carcinogens (Wood, et al., 2001). Recently, it has been hypothesized in many studies that polymorphisms in DNA repair genes reduce their capacity to repair DNA damage and thereby lead to enhanced cancer or other age-related disease susceptibility (Liu, et al., 2007; Povey, et al., 2007).

To date more than 100 DNA repair genes have been identified and their polymorphisms have been reported to be related with some diseases. Among them, polymorphisms of xeroderma pigmentosum complementation group D (XPD) and X-ray complementing group I (XRCC1) have been studied extensively (Clarkson & Wood, 2005; Paz-y-Miño, et al., 2011).

The human XRCC1 (X-ray repair cross-complementing group 1) gene is involved in single strand breaks and base excision repair (BER), it is located on chromosome 19q13.2, encodes for a 633 amino acids protein that plays an important role in BER and single-strand breaks repair (SSBR), following exposure to endogenous ROS or alkylating agents (Padma, et al., 2011; Vidal, et al., 2003; Marsin, et al., 2003). The XRCC1 is a scaffold protein that interacts with other many components of BER as DNA polymerase β, APE1, hOGG1, poly-(ADP-ribose) polymerase and DNA ligase III in the NH_2-terminal, central, and COOH-terminal regions, respectively (Sterpone & Cozzi, 2010). In 1998 Shen et al., described three polymorphisms of XRCC1 gene, which resulted in non-conservative aminoacid changes at evolutionary conserved regions: C → T substitution in codon 194 of exon 6 (Arg to Trp);

G → A substitution in codon 280 of exon 9 (Arg to His) and G → A substitution in codon 399 of exon 10 (Arg to Gln). All these single nucleotide polymorphisms (SNPs) could alter the XRCC1 function and impair DNA repair efficiency or accuracy (Shen, et al., 1998).
Given the large number of polymorphic variants and due to the existence of substantial differences in bladder cancer incidence in different ethnic groups, it is very important determine the frequencies of polymorphisms of many genes in Ecuadorian population affected with bladder cancer. These analyses are of great interest since it allows determining the genetic constitution of the population.

2. Materials and methods

2.1 Biological samples and data collection

A total of 97 formalin-fixed, paraffin-embedded (FFPE) bladder cancer samples were obtained from males and females individuals affected with bladder cancer. These samples were collected from the Department of Urology of Carlos Andrade Marín Hospital in Quito and the Department of Pathology of the Solón Espinoza Ayala Oncologic Hospital of Ecuador (SOLCA). One hundred twenty peripheral blood samples from male and female individuals with a medical history without malignancy served as control. In both cases, all the individuals signed informed consent after receiving information about the study. The study protocol and consent forms were approved by the University Institutional Bioethics Committee.
The distribution of selected characteristics between cases and control groups is summarized in Table 1. As for gender, the group of healthy individuals consisted of 33% of women and 67% of men, while de group of affected individuals consisted of 43% of women and 57% of men. In regard to histological subtype, transitional cell carcinoma accounted for 89%, of total cancer cases; 1% cases consisted of adenocarcinoma, 6% presented urothelial papillary carcinoma and 4% of affected individuals presented squamous cell carcinoma.

Characteristic	Cases Number	Control Number	Odds Ratio
Gender			5.3, 95% CI 2.9-9.5, p<0.001
Women	42	37	
Men	55	83	
Age	71 (>68)	41 (>66)	0.6, 95% CI 0.334-1.020, p<0.05
	26 (<68)	76 (<66)	
Age ($X \pm$ SD)	68 ± 5.5	66 ± 4.5	
Smoking status			23.95, 95% CI 1.28-4.07. p<0.05
Smoker	72	67	
Non-smoker	25	53	
Histotype	Male	Female	
Transitional cell carcinoma	47 (55%)	39 (45%)	
Adenocarcinoma	1 (100%)	0 (0%)	
Urothelial papillary carcinoma	4 (67%)	2 (33%)	
Squamus cell carcinoma	3 (75%)	1 (25%)	

$X \pm$ SD medium ± standard deviation
CI confidence interval

Table 1. Clinical-Pathological characteristic of bladder cancer and control individuals

Concerning cigarette consumption as a risk factor to develop bladder cancer, 74% and 56% of affected individuals and healthy individuals respectively used to smoke, whereas 26% of affected and 44% of controls never smoked.

2.2 Genotyping

The DNA of affected individuals was obtained using the Purelink Genomic DNA extraction kit (Invitrogen, Carlsbad, CA), while, DNA from peripheral venous blood samples was isolated by a "salting out" method (Sambrook, et al., 1989), stored in the nucleic acid data bank of the Biomedical Research Institute at the Universidad de las Américas. The mean concentration of the DNA samples was 80ng/mL measured in a Qubit® Fluorometer (Invitrogen, Carlsbad, CA). We proceeded to study single nucleotide polymorphisms (SNPs) in the GSTP1 (Ile105Val), GPX-1 (Pro198Leu), MnSOD (Ile58Thr) and XRCC1 (Arg399Gln) genes. Genotyping was performed through the polymerase chain reaction–restriction fragment length polymorphism technique (PCR-RFLP).

For GPX-1, MnSOD, GSTP-1 and XRCC1 genes amplification, a PCR final volume of 50µl was prepared, containing 4µl DNA template, 34µl H_2O Milli-Q, 0,4µM of forward and reverse primers, (Table 2) 1.5mM MgCl 2,5µl 10 × buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 0,2µm each deoxynucleotide triphosphate (dNTPs), and 2.5 U Taq DNA polymerase (Invitrogen). For a 191-bp fragment amplification and the analysis of the Pro198Leu polymorphism found in chromosome 3, we used the initial denaturation step lasted 10 min at 95°C, then 35 cycles of 30 s at 56°C, 30 s at 56°C, 45 s at 72°C and 3 min at 72°C were needed. Digestion of PCR product was carried out during 2h at 37°C with the ApaI (Promega, Madison, USA) restriction enzyme. The PCR-RFLP test revealed homozygous individuals (Pro/Pro), (Leu/Leu) or heterozygous (Pro/Leu) (Paz-y-Miño, et al., 2010; Ichimura, et al., 2004). For the amplification of the 145-bp fragment of the Ile58Thr found in chromosome 6, for the PCR reaction, samples were placed in a thermo cycler MJ Research PTC 200® (MJ-Research Inc., Watertown, MA) for the amplification. The initial denaturation step lasted 10 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, 1 min at 72°C, and 10 min at 72°C. For the 177-bp fragment amplification and the analysis of the Ile105Val polymorphism found in chromosome 11, codon 105, exon 5, once the PCR reaction was obtained, the samples were placed in the MultiGene Thermal Cycler TC9600-G for amplification (Labnet, Edison, NJ, USA). The initial denaturation lasted 5 min at 95°C, followed by 35 cycles of 45 s at 94°C, 30 s at 62°C, 30 s at 72°C, and 1 min at 72°C. Digestion of the amplified fragment was performed during 2 h at 37°C with 5 U of the Alw26I

Genes	Primers
GPX-1	Forward, 5′-AAGGTGTTCCTCCCTCGTAGGT-3′
	Reverse, 5′-CTACGCAGGTACAGCCGCCGCT-3′
MnSOD	Forward, 5′-ACTTCAGTGCAGGCTGAACAGC-3′
	Reverse, 5′-CTGGTCCCATTATCTAATAGCTT-3′
GSTP-1	Forward, 5′-ACCCCAGGGCTCTATGGGAA-3′
	Reverse, 5′-TGAGGGCACAAGAAGCCCCT-3′
XRCC1	Forward, 5′-CCCCAAGTACAGCCAGGTC-3′
	Reverse, 5′-TGCCCCGCTCCTCTCAGTAG-3′

Table 2. Sequences of the PCR primers

(Promega, Madison, WI, USA) restriction enzyme. Electrophoresis analysis revealed homozygous individuals (Ile/Ile), (Val/Val) or heterozygous (Ile/Val) (Paz-y-Miño, et al., 2011); whereas for a 242-bp fragment amplification and the analysis of the Arg399Gln polymorphism found in chromosome 19, codon 399, exon 10, the initial denaturation step lasted 5 min at 95°C, then 35 cycles of 45 s at 94°C, 1 min at 59°C, 30 s at 72°C and 3 min at 72°C. Digestion of amplicon was performed during 2 hours at 37°C with the MspI (Promega) restriction enzyme. The analysis revealed homozygote individuals (Arg/Arg), (Gln/Gln) or heterozygote individuals (Arg/Gln) (Wong, et al., 2008).
All the polymorphisms were genotyped using a PCR-RFLP assay. After amplification, PCR products were cleaved by 5U of the corresponding enzyme. After digestion, the fragments were separated by electrophoresis on a 3.0% agarose gel and visualized using ethidium bromide in a transilluminator under ultraviolet light.

2.3 Statistical analysis

All information obtained from the studied individuals was kept in a database and statistical analyses were performed using PASW Statistics 17 for Windows (SPSS, Chicago, IL). The allelic and genotypic frequencies of each single nucleotide polymorphism were calculated from the information provided by the genotypes; and the Hardy-Weinberg equilibrium was determined by using software available on the Internet (http://www.genes.org.uk/software/hardy-weinberg.shtml). Chi-square (X) analysis was performed to determine significant differences between the presence of Ile105Val, Pro198Leu, and Arg399Gln polymorphisms of the studied population. The risk of developing disease in the presence of the studied polymorphisms between affected and control groups was determined using the odds ratio test (OR). Data were analyzed using a 2x2 contingency table.
Table 3 shows the Hardy-Weinberg equilibrium and the genotypic and allelic frequency of the studied polymorphisms. For the GPX1 and MnSOD genes, the genotypic frequencies

Gene	Group	Genotype	Individual (%)	Genotypic Frequency	Allele Frequency
GPX-1	Affected (n = 97)	Pro/Pro	28 (29%)	0.29	0.39
		Pro/Leu	19 (19%)	0.19	
		Leu/Leu	50 (52%)	0.52	061
	Control (n = 120)	Pro/Pro	73 (61%)	0.61	0.79
		Pro/Leu	42 (35%)	0.35	
		Leu/Leu	5 (4%)	0.04	0.21
MnSOD	Affected (n = 97)	Ile/Ile	43 (44%)	0.44	0.68
		Ile/Thr	47 (49%)	0.48	
		Thr/Thr	7 (7%)	0.07	0.32
	Control (n = 120)	Ile/Ile	75 (62%)	0.63	0.82
		Ile/Thr	45 (38%)	0.37	
		Thr/Thr	0 (0%)	0.0	0.18

Table 3. Genotype Distribution and Allele Frequency of the pro198leu and ile58thr

observed in both groups were in Hardy–Weinberg equilibrium (GPX1 cases, X = 0.36, p<0.05; controls, X = 0, p<0.05 and MnSOD; cases, X = 0.02; p<0.05; controls, X = 0.05; p<0.05), confirming that the study samples were obtained from a population in equilibrium. Regarding the GSTP1 Ile105Val polymorphism, we observed that the frequency of the Val allele in control individuals was 0.28 (Table 4). Concerning to the XRCC1 Arg399Gln polymorphism, we observed that the frequency of the Gln allele in control individuals was (0.98) (Table 4). The frequencies of both alleles for the individuals affected with bladder cancer are not shown but according to information reported in other studies could be correlated with the results obtained from the Ecuadorian population.

Genes	Genotype	Genotypic frequency Control	Allele frequency Control
	Ile/Ile	0.54	0.72
GSTP1 Ile105Val	Ile/Val	0.36	
	Val/val	0.10	0.28
XRCC1 Arg399Gln	Arg/Arg	0.01	0.02
	Arg/Gln	0.01	
	Gln/Gln	0.98	0.98

Table 4. Genotypic distribution and allelic frequency of GSTP1 Ile105Val and XRCC1 Arg399Gln polymorphisms

3. Conclusion

Bladder cancer is an important cause of death worldwide, there are many known risk factors for this cancer including age, male sex, smoking habit, and exposure to carcinogens (Pou, et al., 2011). The results obtained from the analysis of four genes using PCR-RFLP technique to determine the presence of the polymorphisms pro198leu in the GPX-1 gene, ile58thr in the MnSOD gene, Ile105Val in the GSTP1 gene and Arg399Gln in the XRCC1 gene in Ecuadorian individuals affected with bladder cancer, although small, support other evidence that genetic polymorphisms of the detoxification enzymes can modify bladder cancer risk.

GSTP1 participates in the detoxification of polycyclic aromatic hydrocarbon in promoting the conjugation of carcinogenic electrophiles with glutathione, thus enhancing excretion in the urine. This gene has been reported to possess two variant alleles. A single base substitution at position 313 of exon 5, guanine for adenine, results in the presence of valine (Val), where originally isoleucine (Ile) was present (Cao, et al., 2005). The prevalence rates of these isoforms are entirely dependent on which ethnic group is being considered (Shimada, 2006). Some have suggested that GSTP1 genes have an increased risk for tobacco-related cancers, including bladder cancer (Souto Grando, et al., 2009). Regarding genetics, the GSTP1 gene encodes proteins that are believed to function in xenobiotic metabolism and play the role as regulator of apoptosis (Moyer, et al., 2008). We found an association between the polymorphism and bladder cancer (data not shown), these findings could be suggesting that the presence of the Val/Val variant could be associated with an increased risk of acquiring detoxification problems, whereas the combination of the Ile/Val and

Val/Val alleles could be associated with the risk of presenting a GSTP1 gene dysfunction. Those individuals presenting the GSTP1 Val/Val and GPX-1 Leu/Leu variables may have a higher risk of acquiring problems in the detoxification (Paz-y-Miño, et al., 2011; Cao, et al., 2005).

Altayli, et al., had reported that smokers with GSTP1 Val105Leu heterozygous genotype had a reduced risk of bladder cancer. Some other authors reported a statistically significant association between the Leu/Leu and Val/Leu genotypes and bladder cancer risk. There are other authors that reported no association between the Ile105Val polymorphism of the GSTP1 gene and laryngeal squamous cell cancer, gastric cancer, and colorectal cancer (Unal, et al., 2004; Cao, et al., 2005).

The GSTP1 Ile105Val polymorphisms appear to be associated with a modest increase in the risk of bladder cancer. Some studies conducted in Asiatic population shows higher risk of developing bladder cancer when GSTP1 Ile/Val and Val/Val versus genotype Ile/Ile were compared, whereas the Chinese population did not have a significant influence on the unadjusted summary odds ratio for GSTP1 Ile/Val and Val/Val compared with GSTP1 Ile/Ile (Ma, et al., 2003). In conclusion, the GSTP1 polymorphisms Ile/Val and Val/Val compared with Ile/Ile seem to be associated with a modest increase in the risk of bladder cancer (data not published).

Our results indicate that the Ile105 allele was associated with an increased risk of bladder cancer. In previous articles, several types of carcinoma have been studied, in which there appeared to be an approximately threefold increase in risk between those with the GSTP1 (Val/Val) allele and those with GSTP1 (Ile/Ile) variant for bladder carcinoma (Harries, et al., 1997).

Successful repair of damaged DNA relies on the coordinated action of many repair enzyme systems. Age dependent decline or imbalance of the activities of the DNA repair enzymes will result in the compromise of the overall capacity of repair for the damaged DNA molecules. Common polymorphisms in DNA repair enzymes have been hypothesized to result in reduced capability to repair DNA damage. XRCC1 is a DNA repair gene that is emerging as an essential element in the repair of both damaged bases and SSBs (Padma, et al., 2011). Additionally, XRCC1 is important in BER, the major repair pathway for nonbulky damaged bases, abasic sites, and DNA single-stranded breaks after treatment with ionizing radiation. Some reports in human populations suggested the 399Gln variant of XRCC1 was associated with greater DNA and chromosomal damage (Yoon, et al., 2011).

It has been suggested that changes in the XRCC1 protein, mainly in amino acid 399, increase the susceptibility for tumor development via genomic instability (Meza-Espinoza, et al., 2009). Nevertheless, another study did not find any effect of the Arg399Gln polymorphisms with regard to DNA damage (Pastorelli et al., 2002), even though it is not well known whether these polymorphisms produce a functional change in the protein. In any case, the risk of cancer depends on the involvement of several factors, and not only on the presence or combination of certain common genetic polymorphisms (Naccarati et al., 2007).

Earlier investigators reported that reduced DNA repair capacity resulting from genetic polymorphism was associated with increased risk for various cancers (Mittal, et al., 2008). In our study the Arg allele was found mainly in the population affected with bladder cancer. In our study, the results obtained show that the XRCC1 Arg399Gln polymorphism, the frequency of the Gln allele was higher in affected individuals when compared to the control group (data not show). Among the polymorphisms of the XRCC1 gene the Arg399Gln

amino acid change alters the phenotype of XRCC1 protein and thereby result in deficient DNA repair. According to our data in case of codon 399 our study exhibited no risk for bladder cancer which was in accord with the Northern Italian population (Shen, et al., 2003). Kelsey et al., 2004 indicated a 40% reduction in risk for bladder cancer among patients with homozygous variant XRCC1 399 (AA) compared with those with wild-type allele carriers. However, Stern et al., 2001 observed contrasting results by showing low risk for AA genotype in bladder cancer patients (OR = 0.7), but with not significant p value. One of the most interesting findings was the obtained by Mittal, et al., 2008 in which XRCC1 codon 399 where AA genotype exhibited 5.27 folds increased recurrence risk (HR=5.27, p=0.04).

On the one hand, GPX1 is suggested to play an important role in moderating H_2O_2 under pathological conditions (Ardanaz, et al., 2010). Over-expression of GPX-1 is associated with a wide range of effects, including the prevention of apoptosis, the protection against toxicity and the reduction of DNA damage (Zhuo, et al., 2009). Given human epidemiological data indicating significant associations between polymorphisms in GPx-1 and the risk of several cancer types due to the important biological activities of the essential trace element selenium are mediated through the function of selenoenzymes (Ichimura, et al., 2004; Hu & Diamond, 2003; Mak, et al., 2006; Choi, et al., 2007; Peters, et al., 2008). In this article we show the relationship between the presence of the Pro198Leu variant of the GPX-1 gene and its association with the risk of developing bladder cancer.

Among the ninety-seven patients analyzed for the GPX1 gene, 28.87% harbored the P/P homozygous genotype, 19,58% were P/L heterozygous and 51.55% were L/L homozygous. Of the 120 controls analyzed for the GPX-1 gene, 60.83% were P/P homozygous, 35% were P/L heterozygous and 4.17% were L/L homozygous (Table 3). For the MnSOD gene in the affected population, 44.33% were I/I homozygous, 48.45% I/T heterozygous, and 7.22% T/T homozygous. For controls 62.5% I/I homozygous, 39.17% I/T heterozygous and 0% T/T homozygous. The allelic frequency of the (I/I) allele was 0.68 for the group of affected individuals and 0.32 for control group (Table 3). The frequencies of the GPX-1 and MnSOD null genotypes were, respectively 39 and 82% in the patients and 79 and 18% in the control group.

When comparing control subjects and those affected with bladder cancer, we found that the presence of the Pro198Leu polymorphism has a relationship with the risk of developing bladder cancer (OR = 3.8; 95% CI 2.1-6.8; $p<0.001$), therefore the presence of the allelic variant (L/L) decreases the unique redox characteristics of the glutathione peroxidase, which can reduce reactive oxygen species and thereby prevent damage of important biomolecules, including DNA, RNA, lipids, proteins, and membranes; reactive oxygen species–induced DNA damage is known to promote tumor progression (Peters, et al., 2008), thereby conferring risk of developing bladder cancer in the Ecuadorian population. Previous studies demonstrate that the carriers of the variant L/L have a four times greater risk of developing bladder cancer than the individuals with the P/P variant (Ichimura, et al., 2004; Hu & Diamond, 2003).

Table 5 shows the respective OR values of the GPX1 and MnSOD genotypes. We found an increased risk of bladder cancer associated with the genotypes for the GPX1 (P/L or L/L) OR = 3.8; 95% CI=2.1-6.8; $p<0.001$), while the MnSOD was not statistically significant (OR = 2.1; 95% CI=1.3-3.5; $p>0.05$). Possible modification of associations between genetic polymorphisms and bladder cancer risk was also achieved by stratifying cases based on old age (OR = 5.3; 95% CI 2.9-9.5; $p<0.001$), sex (OR = 0.6; 95% CI 0.33-1.02; $p<0.05$), and smoking history (OR = 2.3; 95% CI 1.28-4.07; $p<0.05$).

Pro198Leu	Pro/Pro	Pro/Leu	Leu/Leu	Chi-Square	Odds Ratio
Affected	29%	19%	52%	69.9, $p>$ 0.001	3.8, 95% CI 2.1-6.8, p<0.001
Control	61%	35%	4%		
Ile58Thr	Ile/Ile	Ile/Thr	Thr/Thr	Chi-square	Odds Ratio
Affected	44%	49%	7%	0.25, $p > 0.05$	2.1, 85% CI 1.3-3.5, p>0.05
Control	62%	38%	0%		

Table 5. Statistical Analysis

Several studies in different populations worldwide have reported, and an association between these variants with the risk of developing different types of cancer (Raaschou-Nielsen, et al., 2007; Ezzikouri, et al., 2010; Hu, et al., 2010; Arsova-Sarafinovska, et al., 2009; Hansen, et al., 2009). The incidence of these polymorphisms according to the population analyzed, for example: the allelic frequency of L/L in the Japanese population is 0.05, and in the Caucasian population it is 0.36 (Ichimura, et al., 2004; Hu & Diamond, 2003). Furthermore, it has been determined that variants in different populations increases 2.6 times of developing bladder cancer and 1.43 times of developing breast cancer (Ratnasinghe, et al., 2000).

About the age of individuals under study, it has been determined that the risk of acquire bladder cancer is increased in old aged individuals (OR = 0.6; 95% CI 0.334-1.020; p<0.05), and can be considered as a risk factor for developing this disease. Furthermore, it was determined that men are at five times more risk to develop this type of cancer than women (OR = 5.3; 95% CI 2.9-9.5; p<0.001).

Current scientific evidence considers tobacco as a carcinogenic in human, with a causal relationship also to urinary bladder cancer (Lagiou, et al., 2005), being one of the most important risk factors, responsible for almost one-third of bladder cancer deaths (Parkin, 2008). It has been determined that individuals who used to smoke are at two times more risk to develop bladder cancer than individuals that never smoke (OR = 2.3; 95% CI 1.28-4.07; p<0.05). These findings are supported because it has previously been reported that smoking results in lower GPX activity (Ravn-Haren, et al., 2006).

As a result of the ethnic differences, the distribution of the polymorphisms is affected; some studies have found that the risk of developing bladder cancer when a significant incidence of the L/L allelic variant exists, with the proportion of homozygous individuals for the L/L allele being low for the Asian population and high for the Caucasian population (Ichimura, et al., 2004; Hu & Diamond, 2003). These findings have been corroborated for the Ecuadorian population, due to the L/L genotype being present in a high proportion of individuals diagnosed with bladder cancer (n = 51; 51%).

Free radicals, which are produced naturally in the body, can cause oxidative damage of DNA, lipids, proteins and other cell constituents, contributing to the onset of cancer and other chronic diseases (Evans, et al., 2004). Several enzymes, including MnSOD, GSTP, are involved in the scavenging of free radicals and prevention of oxidative damage. MnSOD catalyzes the dismutation of superoxide radicals in mitochondria by converting anion superoxide into hydrogen peroxide and oxygen, being a primary source of defense against cellular oxidants, regulating mitochondrial transport. It plays a key role in protecting cells from oxidative stress, especially in people with a low intake of natural antioxidants (Vineis,

et al., 2007) because low levels of MnSOD gene activity may cause oxidative stress, leading to the development of cancer, diabetes, and neurodegenerative diseases like Parkinson´s and Alzheimer´s (Paz-y-Miño, et al., 2010). Although low expression of MnSOD has often been suggested for different types of cancer, it has been demonstrated that overexpression of this protein inhibited cancerous growth implying it as a tumor suppressor gene (Tamimi, et al., 2004). In addition, MnSOD may exert its effect as a tumor suppressor, by altering pathways involving in cellular apoptosis and proliferation (Canan, et al., 2011).

It has been reported that the frequency of the ile58thr variant of the MnSOD gene does not have a high significance (Hu & Diamond 2003). However there are other reports that have found an association between genetic polymorphisms in MnSOD and myeloid leukemia (Vineis, et al., 2007) indicating that oxidative stress can play a role in cancer. When we compare the values reported with the values obtained in the study we performed, we confirmed that the incidence of the T/T allele maintains a low level within the Ecuadorian population. We have shown statistically that there is no significant difference between the bladder cancer group and the control group (Paz-y-Miño, et al., 2010).

In the same way when we calculate the related risk of this two polymorphism (Pro/Leu and Ile/Thr), a negative relationship between the tendency to develop bladder cancer was found (OR = 2.1; 95% CI 1.2-3.6; $p>0.05$), concluding that the individuals who present the pro198leu variant have an increased risk of developing bladder cancer; contrary to what we found for ile58thr polymorphism and being different from that reported by other authors (Clemente, et al., 2007). It is well known that the ile58thr polymorphism of the MnSOD gene varies within different populations, with an incidence of 11% in Japanese populations and 30% in Chinese populations, compared to the Caucasian populations, which has a 62% of incidence (Hori, et al., 2000; Ambrosone, et al. 1999).

Some authors have reported that the expression level of the manganese superoxide dismutase enzyme, varies within tissues and shows an increment in individuals with brain, skin, lung tumors, breast, bladder cancer and myeloid leukemia (Vineis, et al., 2007; Ichimura, et al., 2004; Clemente, et al., 2007; Ambrosone, et al., 1999), for this reason the present study is important because it allows a characterization of the Ecuadorian population with bladder cancer. Various ethnic groups exhibit significant differences in the distribution of alleles throughout the population, which may influence the interpretation of epidemiological and association studies, these region-specific epidemiological studies provide important information on the frequency of polymorphic allelic variants in various ethnic groups (Souto Grando, et al., 2009).

The very different findings in other populations might be caused by some confounding factors such as ethnicity, selection of control groups and characterization of cases, sample sizes, and gene–gene and gene–environment interactions. In conclusion, these results shown an association with increased risk of bladder cancer in the population studied. In addition, the results suggest that the genotypes of the polymorphisms may be associated with increased risk of bladder cancer.

4. References

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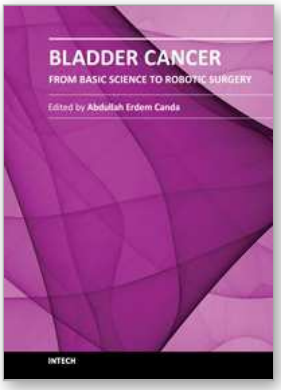

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This book is an invaluable source of knowledge on bladder cancer biology, epidemiology, biomarkers, prognostic factors, and clinical presentation and diagnosis. It is also rich with plenty of up-to-date information, in a well-organized and easy to use format, focusing on the treatment of bladder cancer including surgery, chemotherapy, radiation therapy, immunotherapy, and vaccine therapy. These chapters, written by the experts in their fields, include many interesting, demonstrative and colorful pictures, figures, illustrations and tables. Due to its practicality, this book is recommended reading to anyone interested in bladder cancer.

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