RESEARCH COMMUNICATION

Expression of Xenobiotic Metabolizing Genes in Head and Neck Cancer Tissues

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Abstract

Background: Xenobiotic metabolizing genes are involved in detoxification of carcinogens. Expression of these enzymes may be one of the reasons for interindividual differences in head and neck cancer risks. The aim of current study was first to evaluate the expression of CYP1A1, GSTM1, GSTT1 and GSTP1 and second to observe its relationship with stages of head and neck cancer in Pakistani population. Methodology: Fresh biopsy tissues were taken from oncology institutional hospitals. Semi quantitative reverse transcriptase polymerase chain reaction was used to investigate CYP1A1, GSTM1, GSTT1 and GSTP1 expression in 49 head and neck cancer tumor tissue and 49 normal healthy tissues. Statistical analysis was performed to explore its association with head and neck cancer risk. Results: The current study revealed that the CYP1A1 mRNA expression was markedly reduced in tissues of head and neck carcinoma compared to adjacent normal tissue (OR 4.5, CI 1.5- 13.4). CYP1A1 expression was downregulated in 62.5% tissues of stage 1, 72.7% tissues of stage 2, 60% tissues of stage 3 and 100% tissues of stage 4. Undetectable or partial loss of expression of GSTM1 and GSTT1 mRNA was also observed at a higher rate in head and neck cancer tissue compared to control (OR 4.5, CI 1.5- 13.4 and OR 3.2, CI 1.1- 9.6 respectively). GSTM1 and GSTT1 expression was also downregulated in stage wise pattern; stage 1 had 50% and 12.5% tissues showing down regulation of GSTM1 and GSTT1 genes respectively, both GSTM1 and GSTT1 had 55% tissues with down regulation in stage 2, similarly stage 3 had 60% tissues showing down regulation of these genes and stage 4 had 86% and 71% tumors. GSTP1 mRNA expression was significantly higher in cancer tissue as in control tissue (OR 4.2, CI 1.2- 15.3). GSTP1 over expression also revealed related to stages with 36.4%, 60% and 71% tumor of stage 2, 3 and 4 respectively. Conclusion: Our results revealed that CYP1A1, GSTM1 and GSTT1 are downregulated in the head and neck cancer progression while GSTP1 is upregulated. These down regulations and up regulation were more marked in advanced stages of head and neck cancer. Therefore, CYP and GST expression may be an important mechanism involved in the carcinogenesis but the underlying mechanisms leading to such regulations in expression deserve further investigations.

Keywords: GSTM1 - GSTT1 - GSTP1 - CYP1A1 - expression - mRNA - head and neck cancers

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Introduction

Factors for determining individual's susceptibility to head and neck cancer are still largely unknown. One possible explanation for this variation may be an imbalance in the detoxification enzymes. Phase I enzymes like cytochrome P 450 or Phase II enzymes such as glutathione S transferases may be altered probing an increase in head and neck cancer risk. Cytochrome P 450 1A1 (CYP1A1) is a heme containing mono-oxygenase that is involved in the metabolism of endogenous and exogenous compounds (Gonzalez and Gelboin, 1994). CYP1A1 is involved in the formation of aryl hydrocarbon hydrolase enzyme that is responsible for the activation of many polycyclic aromatic hydrocarbons and aromatic amine (Bartsch et al., 2000). It is implicated in the metabolism of benzo[a]pyrene, a potent tobacco carcinogen. Marked enzymatic activities of CYP1A1 have been demonstrated in human head and neck cancer epithelium (Farin et al., 1995). CYP1A1 transcription depends on aromatic hydrocarbon receptor and aromatic hydrocarbon nuclear transporter gene products (Nebert, 1989). CYP1A1 gene transcription is induced on CYP1A1 promoter site when aromatic hydrocarbon receptor is bound to its chemical ligands and is transported in the nucleus by aromatic hydrocarbon nuclear transporter.

GSTs present a family of soluble isoenzymes that play an important role in detoxification process. They catalyze the nucleophilic addition of glutathione to lipophilic electrophiles produced by phase I enzymes and mark the first step of carcinogen elimination (Chasseaud, 1979). GSTs are expressed in tissue specific manner (Tu et al.,1983) most of the GSTs are in liver, muscles, brain, testes, heart, blood and upper aerodigestive mucosa (Hayes and Pulford, 1961; Tsuchida, 1990; Matthias et al., 1998; Matthias et al., 1999). The fact that most of

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the xenobiotic enzymes are expressed in a tissue specific manner leads to great differences in the activation and inactivation of xenobiotics in different tissues. Hereditary differences in the expression and activity of human GSTs have been reported and low enzymatic activity of GSTs was associated with lung cancer (Seidegard et al., 1986). GSTM1 and GSTT1 reduced expression is due to null genotype of these genes (Lafuente et al., 1993; Lafuente et al., 1995). GSTP1 is over expressed in many cancer like stomach, bladder, colorectal, oral, pharynx, larynx, skin, lung and breast cancer and the high levels of GSTP1 may contribute to drug resistance (Tsuchida et al., 1989; Niitsu et al., 1989; Tanita et al., 1993; Hayes and Pulford, 1995).

Therefore, investigation related to the expression of CYP1A1, GSTM1, GSTT1 and GSTP1 in correlation to stages of head and neck cancer in tumor and control tissue was conducted.

Materials and Methods

Total 49 unrelated head and neck cancer patients undergoing surgery at Pakistan Institute of Medical Sciences (PIMS) Islamabad, Allied Hospital Faisalabad and Military Hospital Rawalpindi were recruited between 2009 and 2010. The study was approved from ethical committees of hospitals and university. All patients were diagnosed with carcinoma of head and neck by cytological, imaging and histopathological examinations. Informed consent was obtained from patients prior to surgery and interviewed. Clinical data were available for all the specimens tested. Tumor tissue specimen paired with their corresponding adjacent normal tissue were surgically obtained and collected in RNA later (Ambion).

RNA isolation

Total RNA was extracted from tissue with TRIZOL (Invitrogen, USA) according to the manufacturer's protocol as described previously (Zhong et al., 2006) and was stored at -80˚C until further use. RNA was quantified by spectrophotometry and equal amount of RNA for all the samples was used for subsequent use.

cDNA synthesis and semi-quantitative reverse transcriptase polymerase chain reaction analysis

Total RNA isolated from controls and tumor tissue was analyzed for CYP1A1, GSTM1, GSTT1 and GSTP1 mRNA by semi-quantitative reverse transcriptase-PCR. cDNA was prepared from total RNA as described in manufacturer's protocol (Invitrogen, U.S.A.) and 2 µl of RT product was used for subsequent PCR reactions. Primers were synthesized from molecular biology products with sequences described previously (Fusako et al., 2004; Ioanna et al., 2001). Prior to amplification of CYP1A1, GSTM1, GSTT1 and GSTP1 normalization was carried out with β-actin, the housekeeping gene.

Electrophoresis

Aliquots of the PCR reaction were subjected to electrophoresis on 2% agarose gels and PCR fragments were visualized by ethidium bromide staining and photographed on gel documentation system. The mRNA expression of the housekeeping gene was used as a quality control for the samples showing equal cDNA in all samples.

Statistical analysis

Odds ratio and 95% confidence interval was used to evaluate the significance of results. Statistical analysis was performed by SPSS (8.0) software.

Results

The 49 head and neck cancer patients whose tissue specimens were used had a mean age of 51.6 (+16.1) years. Male to female ratio was 2:1 with most frequent area of cancer being larynx followed by oral cavity and pharynx in a ratio of 3:2:1 respectively. Latter stages of head and neck cancer had higher mean ages compared to earlier stages. Mean age of stage 1 head and neck cancer was 45(+12.9) years, stage 2 was 47.7(+16) years, stage 3 was 53.4(+18.6) years and stage 4 was 60.6(+12.6) years. β actin was used as a control, housekeeping gene for uniform expression of mRNA (Figure 1).

CYP1A1 expression in head and neck cancer

Reverse transcriptase PCR was used to detect expressional variation of mRNA between tumor and adjacent normal tissue for CYP1A1. It was found that CYP1A1 was significantly downregulated in head and neck tumor tissue compared to control tissue (OR 4.5, CI 1.5-13.4). However β actin showed uniform expression with all the samples showing equal amount of cDNA in all the samples (Figure 1).

CYP1A1 mRNA expression also revealed a stage specific pattern where downregulation was at a higher rate in latter stages of head and neck cancer compared to early stages. CYP1A1 expression was downregulated in 62.5% tumor tissues of stage 1, 72.7% tumor tissues of stage 2, 60% tumor tissues of stage 3 and 100% tumor tissues of stage 4.

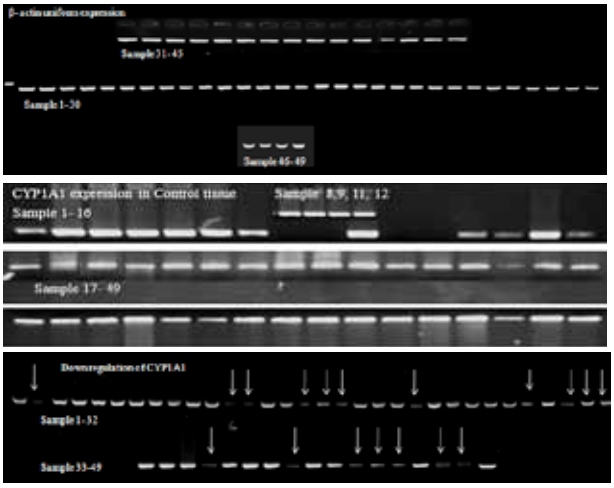

Figure 1. Semiquantitative PCR of Housekeeping Gene β- actin (A) CYP1A1 mRNA Expression in Control (B) and Tumor (C) Tissue

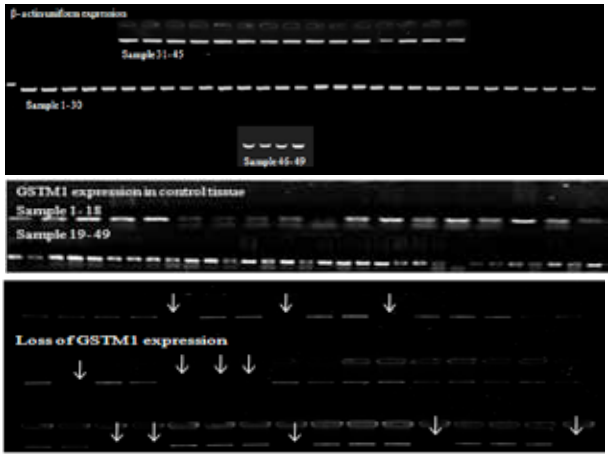

Figure 2. Semiquantitative PCR of Housekeeping Gene β- actin (A) GSTM1 mRNA Expression in Control (B) and Tumor (C) Tissue

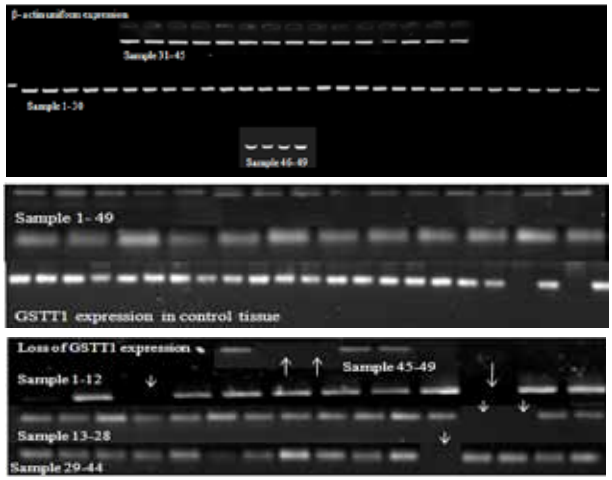

Figure 3. Semiquantitative PCR of Housekeeping Gene β- actin (A) GSTT1 mRNA Expression in Control (B) and Tumor (C) Tissue

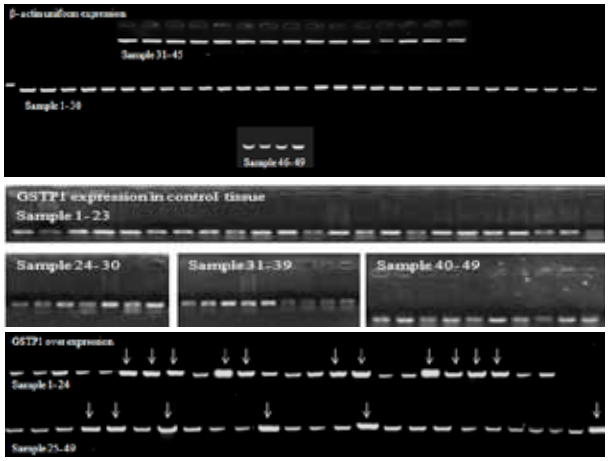

Figure 4. Semiquantitative PCR of Housekeeping Gene β- actin (A) GSTP1 mRNA Expression in Control (B) and Tumor (C) Tissue

GSTM1 expression in head and neck cancer

Results of GSTM1 mRNA expression found an undetectable or partial loss of expression (Figure 2). Undetectable or partial loss of expression was observed in control and tumor specimens but at a significantly higher rate in tumor tissues (OR 4.5, CI 1.5- 13.4). GSTM1 downregulation due to undetectable or partial loss of expression showed an increase with latter stages of head and neck cancer tumor. Stage 1 tumor tissue had 50% tissues showing downregulation, stage 2 had 55%, stage 3 had 60% and stage 4 had 86% tissues with downregulation.

GSTT1 expression in head and neck cancer

Undetectable or partial loss of expression of GSTT1 mRNA was also observed at a higher rate in head and neck cancer tissue compared to control tissue (OR 3.2, CI 1.1- 9.6) (Figure 3). GSTT1 expression was also downregulated, due to undetectable or partial loss of expression, in stage wise pattern; stage 1 had 12.5% tissues showing down regulation of GSTT1 gene, GSTT1 had 55% tissues with down regulation, similarly stage 3 had 60% tissues showing down regulation of GSTT1 genes and stage 4 had 71% tumor tissues with downregulation.

GSTP1 expression in head and neck cancer

In contrast to downregulation of CYP1A1, GSTM1 and GSTT1 mRNA expression, GSTP1 expression was upregulated. GSTP1 mRNA expression was significantly upregulated (Fig 4) in head and neck tumor tissue compared to control tissue (OR 4.2, CI 1.2- 15.3). GSTP1 over expression also appeared related to stages with no upregulation in stage 1 whereas, 36.4%, 60% and 71% tumor of stage 2, 3 and 4 respectively showed upregulation suggesting an increase in over expression with latter stages of head and neck cancer.

Discussion

Carcinogens are detoxified by phase I and phase II enzymes following two pathways; either the phase I enzymes detoxify the carcinogen or convert them into more electrophilic compounds. These intermediate forms are identified by phase II enzymes, detoxify and eliminate from the body. Impairment in these enzymes lead to altered DNA structure (Gelboin, 1980). Variations in the expression of CYP1A1 and GSTs like GSTM1, GSTT1 and GSTP1 could potentially explain the differences in susceptibility to the carcinogenic effects leading to head and neck carcinoma. CYP and GSTs expression varies in different tissues therefore detoxification of carcinogens by locally expressed enzymes may be an important determinant of carcinoma rather than from more distant tissues or serum levels of these enzymes (Strange et al., 1984; Shea et al., 1988). Different studies assessed the risk of head and neck cancer in relation to CYP1A1, GSTM1, GSTT1 and GSTP1 genotypes (Matthias et al., 1998; Morita et al., 1999; Sato et al., 2000). Interindividual variations in the expression of CYP and GSTs are dependent on genotype as well as post transcriptional factors that may be tissue specific (Smart and Daly, 2000; Anttila et al., 2001; Wandel et al., 2000). Therefore the most accurate method to determine the effects of CYP and GSTs on carcinogens is to evaluate the expression in the tissues of interest associated with head and neck carcinoma.

It was found that the expression of CYP1A1 was

significantly downregulated in tumor tissues than in the adjacent control tissue. These results were in accordance with the previously published studies in different carcinomas such as breast cancer (Ei et al., 2003). CYP1A1 has been found to be actively present in human placenta (Whyatt et al., 1995), duodenum, jejunum (Pavek et al., 2008) skin and keratinocytes (Conway et al., 2009). Studies had found variability in the levels of CYP1A1 transcript in human lung cancer (Wei et al., 2001; Chang et al., 2007). Murray and colleagues had drawn associations regarding the differential expression of CYP1A1 in non-cancerous and esophageal cancerous tissues. CYP1A1 enzyme expressed variation in esophageal carcinomas and control tissue (Murray et al., 1994). CYP1A1 were further detected in 68% of the urinary bladder tumors and their expression correlated with bladder tumor grade (Murray et al., 1995). However in head and neck cancer it was found that CYP1A1 was highly expressed in control tissue compared to tumor and also correlated to tumor stage. CYP1A1 expression is controlled via AhR pathway when benzo pyrene activates CYP1A1 (Hildebrandt et al., 1981). The increase in CYP1A1 activity leads to H2O2 production (Pompon et al., 1997) and thus it leads to transactivation of NFI. NFI activation in turn repress CYP1A1 expression (Morel and Barouki, 1998; Paton and Renton, 1998).

Expression levels for GSTM1 and GSTT1 were lower in head and neck cancer tumor tissue compared to control tissue. Under expression of GSTs had also been reported previously in head and neck cancer (Theo et al., 1995). Loss of GSTM1 and GSTT1 expression had also been found in breast and prostate cancer (Ioanna et al., 2001; David et al., 2007). Possible explanation for this down regulation may be due to genetic polymorphism causing null genotype of GSTM1 and GSTT1 genes in study population. Approximately 50% of Caucasian population is GSTM1 and GSTT1 deficient (Xu et al., 1998). Our previous study also found significantly higher rate of GSTM1 and GSTT1 gene deletions in similar study group (Nosheen et al., 2010). Therefore the current study further strengthens the concept that high rate of null genotype was responsible for subsequent loss of expression of these genes. Null genotype of these genes do not express in RT-PCR. Few studies had reported slight expression of GSTM1 which may be due to other isoforms of GSTM (Diemut et al., 2004).

GSTP1 was over expressed in tumor tissues compared to adjacent control tissue in the current study. Similar results in head and neck cancer have been reported in literature (Tanita et al., 1993; Chen and Lin, 1995; Bentz et al., 2000). GSTP1 is over expressed in many cancers such as stomach, bladder, colorectal, lung, ovarian, skin and breast (Tanita et al., 1993; Mulder et al., 1995; Hayes and Pulford, 1995). GSTP1 over expression is not related to genotype but probably transcriptionally regulated. GSTP1 over-expression may be due to a number of different mechanisms including gene amplification, transcriptional activation, protein stabilization, and genetic abnormalities (Matthias et al., 1998). Increased levels of GSTP1 may be occasionally involved in the intrinsic drug resistance of head and neck cancers. However, silencing of this gene had been shown to increase tumor sensitivity to same drug. Lower expression of GSTP1 may be associated with better response to chemotherapy and improved prognosis (Isabelle, 2010). GSTP1 upregulation is considered as a risk for cancer progression because it inhibits apoptosis by reacting with cJUN (Wang et al., 2001; Holley et al., 2007). GSTP1 gene expression is known to be regulated with several transcriptional mechanisms like Sp1 (Moffat et al., 1996), AP1 (Xia et al., 1996), retinoic acid response element (Lo et al., 1997) and PKA/CREB1 (Lo et al., 2002). But the best possible regulating mechanism may be mediated by differential methylation of GSTP1 promotor (Antoun et al., 2000; Lee et al., 1997).

In conclusion, expressional variation of CYP1A1 and GSTs was reflected showing downregulation of CYP1A1, GSTM1 and GSTT1 and up regulation of GSTP1 in head and neck cancer. A correlation with stages of cancer was also found with increased upregulation and downregulation at advanced stages of head and neck cancer. However to explain them as prognostic markers for staging of head and neck cancer needs further studies in order to explore more about these genes and head and neck cancer.

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