The Journal of Laryngology & Otology
May 2002, Vol. **116,** pp. 352–354

Expression of GSTM1 in angiofibromas

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Abstract
The aetiology of juvenile nasopharyngeal angiofibroma (JNA) is largely unknown. In this study, we have investigated the expression of glutathione S transferase M1 (GSTM1) gene in angiofibroma.

The GSTM1 allele gene locus is normally found in all human beings. When this is not expressed there is an increased risk of developing a malignancy of the upper aerodigestive tract. In this study, we have assessed eight samples of JNA for the expression of GSTM1, by the polymerase chain reaction (PCR) followed by polyacrylamide gel electrophoresis (PAGE), three of the eight patients studied failed to express this gene. Further investigation in this area is warranted.

Key words: Angiofibroma; Glutathione Transferase; Genetic Markers

Introduction

Juvenile nasopharyngeal angiofibroma (JNA) is an uncommon benign tumour of adolescent males.[1–3] They typically arise from the lateral wall of the nasopharynx[4] and present with nasal obstruction and epistaxis.[3] Many studies have been carried out to discover the aetiology of this condition, but it still remains obscure.[5] There are a few reports examining the possible role of tumour promoters.[6,7] In this study we have tried to find out whether there is any correlation between the expression of the GSTM1 gene and JNA.

This tumour occurs most commonly in adolescent males, although rarely it has been reported in females.[8,9] It regresses in some while progresses in others.[4,10,11] There are rare but definite reports of the tumour being malignant in a few cases.[12] All these findings suggest a genetic change occurring in the tumour. Our aim was to find out if the rate of expression of GSTM1 gene, a well-known cytoprotective gene,[13,14] was altered.

Materials and methods

Eight patients with JNA presented with a history of epistaxis and nasal obstruction. Contrast enhanced computed tomography (CT) scan was done in all the patients which showed the presence of a brilliantly contrast enhancing mass in the nasopharynx with widening of the sphenopalatine foramen. Pre-operative digital subtraction angiography (DSA) carried out in all the eight patients showed the main feeder to be from the ipsilateral internal maxillary artery and they were subsequently embolized to reduce the intra-operative blood loss.

These eight patients then underwent surgical excision of the tumour. On removal, a small piece of the tumour tissue was stored immediately in the freezer compartment of the refrigerator. This was then transported in ice to be stored at −80°C.[7] All these steps are vital to maintain the structure of the DNA by preventing degradation. From this sample, the DNA was extracted later by a standardized method called the phenol chloroform method.[15]

Firstly the cells were lysed in cell and nuclear lysing solution in the presence of proteinase K solution. Subsequently, the DNA was isolated from this by subjecting to phenol and then phenyl chloroform (two washes) and then finally was washed in chloroform. Each time the aqueous phase was mixed thoroughly. After washing with chloroform the aqueous phase was mixed with 100 per cent ethanol to precipitate DNA. This DNA was then solubilized in water and subjected to the polymerase chain reaction (PCR).

The PCR was carried out to assess the genetic polymorphism of the GSTM1 locus, with the GSTM2 locus used as an internal control. The reaction was carried out in the presence of primers for GSTM1 and GSTM2 genes in the presence of dimethyl sulfoxyl (DMSO), PCR buffer (GIPCO-BRL), magnesium chloride 1mMole, deoxy-nucleotide triphosphates (dNTPs) and distilled water. Primers and Taq were added after denaturation at 94°C.

The extracted DNA was incubated in the thermal cycler together with the above reagents. The thermal cycle was as follows:

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Accepted for publication: 21 November 2001.

(1) 94°C – 10 minutes – to denature the DNA.
(2) 85°C – 5 minutes – for primer and Taq to act
(3) 59°C – 1 minute – produces annealing.
(4) 94°C – 1 minute and { 30 cycles of each. denatures and anneals and promotes …
(5) 59°C – 30 seconds – polymerization.

Then at 72°C for 5 minutes, primer extension was achieved. This product was kept at the holding temperature of 15°C.

The PCR product was then run on 12 per cent polyacrylamide gel electrophoresis and was visualized under UV transillumination after staining with ethidium bromide. As is known, the GSTM1 gene product is of 270 base pairs (bp) while the GSTM2 product is of 160 bp.[16]

The pattern obtained was photographed and is shown in Figure 1.

Observations

The samples from eight patients of clinically, radiologically and histologically confirmed JNAs were collected for the present study conducted between September 1999 to October 2000.

All eight patients were males belonging to the adolescent age group. Two patients were from the northern part of India while the remaining six were from Maharashtra state. All were students by occupation. None of them smoked or used tobacco in any other form. All of them belonged to a low socioeconomic group. None of the patients had any other family members suffering from similar disease.

All the patients had history of nasal obstruction and epistaxis. In addition three of them also complained of persistent rhinorrhoea and four patients had change of voice (rhinolalia clausa). The four patients who had rhinolalia clausa also complained of snoring at night. Anterior rhinoscopic examination revealed a soft pinkish mass in the nose in six out of eight patients. A palatal bulge was seen in four patients. All the patients were treated surgically.

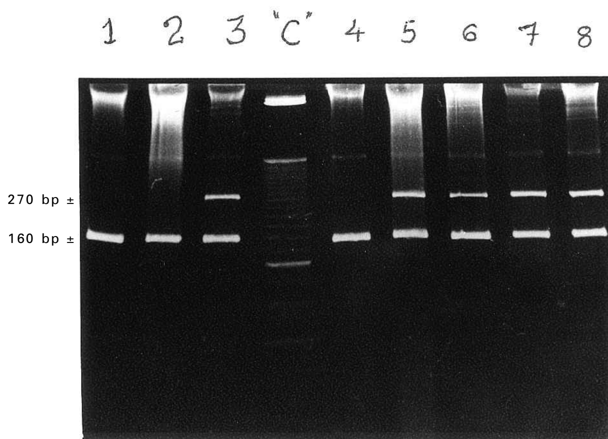

Fig. 1

2D gel electrophoresis clearly showing the absence of expression of the GSTM1 gene product in three out of eight samples. 'C' indicates control well. '270 bp' indicates GSTM1 and '160 bp' indicates GSTM2 products. bp = base pair.

On subjecting the tumour tissue to PCR, all the samples showed expression of GSTM2 gene, which was used as a housekeeper gene in this experiment ie. this gene was used as an internal control for the reaction. As regards the GSTM1 gene product, only five out of the eight patients expressed the gene product while three of them did not express it. This translates into a percentage of 37.5 per cent containing the null genotype for GSTM1.

Discussion

JNA is a well known but rare tumour of the adolescent male,[13,17,18] occurring more commonly in the Near and Far East than in Americans and Europeans.[2] The incidence as reported by various authors stands at an average of 0.05–0.5 per cent of all head and neck tumours[6] or 5000–50000 of all ENT consultations.[5,13,14] Since our hospital is a tertiary care referral centre, we received as many as eight patients in a short span of one year giving us an incidence of almost 1 in 10 000 ENT consultations. Even though the treatment modalities for the various stages of the disease have been well established,[2,19] the root cause of this disease is still poorly understood.[1,5] There have been efforts in the past to look for the expression of p53 gene product,[7] but nothing conclusive has been arrived at. There have also been many investigations to find out the involvement of various hormonal receptors[20] – again with inconclusive results.

The fact that the tumour arises predominantly in males,[1–3,8] regresses on its own after a particular age at least in some patients[4,10,11] and in a rare few degenerates into malignancy,[12] points to the fact that there might be genetic interplay involved. Moreover, it is well known that for a tumour to arise, due to whatever reason, there has to be some kind of genetic derangement occurring in the tumorigenic tissue. Oncogenes and tumour-suppressor genes together may represent the two most important genetic elements in neoplasia,[21] ie. unless there is a lack of suppression or there is a promotion of growth at the genetic level, a tumour cannot grow irrespective of the fact whether it is benign or malignant.

It is known that smoking produces its harmful effects by being a pollutant – a self-pollutant. It is a well-known fact that the non-expression of the GSTM1 gene predisposes a person to upper aerodigestive tract malignancies – especially so if the person happens to be a smoker.[13,14] This is because of the fact that the GSTM1 gene produces an enzyme, that acts as a detoxifying agent thereby playing the role of a cytoprotective substance.[13,14] The absence of the GSTM1 gene (null genotype) product means the loss of this cytoprotection, thereby predisposing the person for tumour development.

All patients had been exposed to smoke from cooking fuel (wood or wood oil) but none smoked. The other air pollutants in the environment can also produce harmful effects if a person lacks the detoxifying enzymes.

https://doi.org/10.1258/0022215021910997 Published online by Cambridge University Press

There was no history of similar illnesses in the family. This can happen if the gene is carried by one parent in whom the mutant allele is not penetrant or if there is non-paternity (which is seen in three to five per cent in many cultures[22]). Even in a disease with genetic aetiology, it is possible not to have any family history, if the disease is due to a fresh mutation or if the genetic cause is multifactorial.[22] Again multifactorial aetiology is thought to be important for many diseases that develop after adolescence, and diseases with later age of onset have decreased heritability on average.[22] Also, if the disease were to kill the patient before he reproduces, or if it kills the ability to reproduce, then the family history will be difficult to obtain especially when the incidence rate is so low.[22]

The GSTM1 gene product was not expressed in three out of eight samples. It translates into approximately 37.5 per cent of the samples expressing the null genotype of GSTM1. The result is significant because approximately only 17 per cent or less of the normal Indian population will express the null genotype[23] whereas in the present study it was much higher than this figure (37.5 per cent). Again here the results can be accepted as true results because of the fact that all eight samples ie. 100 per cent showed the GSTM2 gene product, which served as the internal control.

It is possible that this non-expression of the GSTM1 gene is one of the factors responsible for the causation of the tumour along with various still unknown factors. Only further similar studies in the future can tell us more about it. The present result may not be statistically significant, considering the small test group of eight patients. But it is almost impossible in such rare diseases to have a statistically significant study group, unless the test is standardized and is conducted across geographical boundaries. One other way of knowing the statistical significance of the result will be through meta-analysis of the world literature.

Conclusion

The cytoprotective gene GSTM1 was not expressed in 37.5 per cent of the cases of JNA examined. This result cannot be taken to be conclusive, but it warrants further investigation.

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Dr K. Gautham takes responsibility for the integrity of the content of the paper.
Competing interests: None declared

https://doi.org/10.1258/0022215021910997 Published online by Cambridge University Press