Evaluation of Chromosomal Disorders in Tissue and Blood Samples in Patients with Oral Squamous Cell Carcinoma

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Statement of Problem: Many studies have indicated that genetic disturbances are common findings in patients with Oral Squamous Cell Carcinoma (OSCC). Identification of these changes can be helpful in diagnostic procedures of these tumors.
Purpose: The aim of this study was to appraise the chromosomal disorders in blood and tissue patients with OSCC.
Methods and Materials: In this descriptive study, the study group consisted of all OSCC patients who were referred to the Faculty of Dentistry, Tehran University of Medical Sciences, Maxillofacial Surgery Clinic of Shariati Hospital, and Amir Aalam Hospital from September 2000 to November 2002. In order to study chromosomal disorders in the peripheral blood lymphocytes, 5 mL of blood was obtained from each patient In patients with the large lesion, a piece of involved tissue were obtained and cultured for 24 hours. This led to 29 blood samples and 16 tissue specimens and any relation between OSCC and age, sex, smoking and alcohol use were evaluated.
Results: In this study, OSCC was more common in males than in females (3 to 5). 31% of our patients were smokers, and one had a history of alcoholic consumption. There was an increase in incidence of OSCC with age. In this study, all patients had numerical (aneuploidy, polyploidy) and structural chromosomal disorders (double minute, fragment, breakage and dicentric). There was significant difference between blood and tissue chromosomal disorders (aneuploidy, polyploidy,breakage) in OSCC patients.
Conclusion: It can be concluded that chromosomes in patients with OSCC might show some genetic aberration and evaluation of involved tissue might be better way for determining this disorders.

Key Words: Oral squamous cell carcinoma; Chromosomal disorders; Culture Cell

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Cancer is one of the most complicated defects that clinical medicine is facing today, and its treatment at an advanced stage is mostly ineffective and its cure can be considered as a miracle. However, at an early stage, and effective treatment can be given with less suffering of the patient. Therefore, its detection at an early stage and identification of individuals prone to it is crucial.

In the past, it was believed that viruses and environmental factors were the cause of cancers, but it is now clear that the main causes are genetic mutations and carcinogenic factors affecting cell's DNA.(1)

Indeed it may be claimed that carcinogenesis causes serial mutations that result in irregular

growth of clonal cells, followed by malignant growth in a part of the body.[2] In recent years, biological molecular techniques have been able to change our views about diagnosis and treatment of neoplasm. It is important for the patient's survival, because when a tumor recurs, it become more invasive, as in a study on breast cancer Haffty showed obvious decreasing in 5 years survival of patients with recurring tumors in comparison with patients with primary tumors.[3]

Today it is obvious that genetic disturbances are common in head and neck Oral Squamous Cell Carcinoma (OSCC), and identification of these may be useful in follow- up and prognosis determination of these tumors. [4]

There is evidence that, in most neoplasms, malignant cells have chromosomal disorders- (such as deletion, reciprocal translocation or trisomy).[5] Common chromosomal disorders are created during growth of the neoplasm, for instance in Chronic Myelogenic Leukemia (CML). [5]

There are two kinds of gene that are implicated in cancer, oncogenes and tumor suppressor genes. They have opposing effects in cancer creation.[1]

Oncogenes facilitate cells to become malignant, while tumor suppressor genes inhibit growth of cancers. Oncogenes are seen in a cell as proto-oncogenes. In contact with retroviruses or mutant agents, they become active. Chromosomal translocation is a common mechanism for activating proto-oncogene OSCC.

OSCC is the most common oral malignancy (greater than 90%). Its characteristic is to invade supporting connective tissue. This malignancy is able to invade the lymph node and metastate to distant organs. 80% of OSCC is seen in smokers[6].Also alcohol use, HPV, specially HPV 16 or HPV 18, HSV, Candida Albicans, pernicious anemia and immunity defects are suggested factors for its etiology .[6-8]

Most patients are older than 45 years of age. However, the behavior of this tumor in the young is more invasive. [6]

Studies of cell clones have shown chromosomal disorder in numbers 1, 3, 4, 8, 14, and 15. On the basis of previous studies deletion areas were related to tumor suppression genes, and amplified areas were related to oncogenes.[9]

Hermsen et al by use of cytogenic analysis on 11 OSCC patients observed that chromosomal disorders were present both numerical and structural, in all tumors. [10]

Ita and Okafuji studied 11 squamous cell carcinomas by Comparative Genomic Hybridization (CGH). They observed that chromosomal disorders are more obvious in stage II than other stages. [11]

The aim of this study was to describe the chromosomal disorders in blood and tissue of patients with OSCC.

Materials and Methods

In this descriptive study, the study group consisted of all OSCC patients who were referred to the Faculty of Dentistry, Tehran University of Medical Science, Maxillofacial Surgery Clinic of Shariati Hospital, and Amir Aalam Hospital from September 2000 to November 2002.

In order to study chromosomal disorders in the peripheral blood lymphocytes, 5 mL of blood was obtained from each patient In patients with the large lesion, a piece of involved tissue were obtained and cultured for 24 hours. This led to 29 blood samples and 16 tissue specimens and any relation between OSCC and age, sex, smoking and alcohol use were evaluated.

For chromosomal study all kind of cells can be used with the exception of red blood cells, which lack nucloids. Today peripheral blood lymphocytes and cell cultures are used for chromosomal study of adult individuals.

Culture of Samples: Eight drops from the blood sample were mixed with heparin and cultured in RPMI 1640 enriched with 20% FCS. 0.1 mL phitohemaglutinin (PHA) was added to

the culture. This was carried out in sterile environment, and the sealed test tube. Then cultured samples were placed in a carbon dioxide incubator at 37°C. Two cultures were prepared, and were incubated for 72 hours. Every 24 hours, the cultures were checked, and if any pollution was observed, that particular culture was rejected, and the exercise was repeated.

Sixty-eight hours after culturing, 0.004% colshicin was added to each sample. After 72 hours, the samples were extracted form the incubator and centrifuged at 800 rpm for 5 to 7, minutes. The light solution was extracted by Pasteur pipette, and then 8 mL of Potassium Chloride hypotonic solution were added to the sample, and placed in a Benmarry at 32°C for 10-12 minutes. The samples were centrifuged again at 800 rpm for 5-7 minutes, and the solution again extracted. The sample was fixed slowly with a fixative agent. Twenty-four hours after this, the sample was centrifuged, the solution extracted, and this process repeated until a white suspension remained. This suspension was then dropped onto previously cooled slides. The slides were stained with gimsa and studied under the microscope.

Banding Methods: These methods can be used to show different disorders, such as translocation, deletion and insertion. This study used G-banding to show chromosomal disorders in blood.

G-Banding Method: We first prepared a solution of 3% tripsine in a phosphate buffered solution (FBS). This solution was diluted to 7 parts FBS, 3 parts tripsine. The slides were placed in this tripsine solution for 4-5 minutes dipped in distilled water, and then stained by gimsa for 2-4 minutes.

Observation of Microscopic Slides: All areas of each slide were systematically searched for chromosomal disorders. Their locations were recorded, and photographed.

Tissue Sampling: The obtained tissues from patients with OSCC placed in sterile tubes containing RPMI. 1640 culture environment with antibiotic and then were transferred to Genetic Department in Razi Institute.

The samples were crushed completely inside especial tubes contained 0.3% tripsin solution by sterile instruments and scissors, and then inserted on magnetic stirrer for ten minutes. Afterwards the cells that separated completely carried out by sterile pipette to a culture contained FCS, and this process repeated at least three times until collecting enough tissue cells.

Then environment contained separated cells were centrifuged at 800 rpm for several times. 1×10^4 cells were added to 7 mL of RPMI 1640 with 20% FCS in dishes with 20 cc capacities, and were placed in a 5% carbon dioxide incubator. After one week when tissue cells looked like spindle stick to the floor of glasses and were developed, 0.4% colshicin was used to stop metaphasic cells. Spindle cells pulled off by 0.3% tripsin solution after three hours then 0.75 m kcl hypotonic solution was used at 37° to swell cells.

These cells were centrifuged at 800 rpm for 10 minutes. Then these cells were fixed by carnow (Methanol Acetic Acid) fixative agent. Afterwards slides were prepared and different staining methods were used to study chromosomal disturbances.

Statistical Methods: chromosomal disorders in blood and tissue groups were determined and reported. Comparison of chromosomal disorders in blood and tissue samples were analyzed by paired t-test.

Results

This study accomplished on 29 patients with OSCC. For assessment of their chromosomal disorders, 50 chromosomal metaphases were studied. The patient with OSCC who were conducted blood samples were called group I and the patient with OSCC who were conducted tissue samples were called group II.

The group I was composed six (20.7%) females

and 23 (79.3%) males and group II were conducted 4 (25%) females and 12 (75%) males.

Table I shows the age distribution of patients in groups I and II. Nine (31%) and four (25%) patients of group I and II were smokers respectively. One of patient (3.4%) in group I and none of group II had history of alcohol consumption. These disorders were observed in the majority of chromosomes and were not related to a specific one.

Table II shows Pair t test in chromosomal disorders in blood and tissue patients with oral SCC.

Discussion

In this study we found that OSCC in males is more common than females (3 to 5 fold) and this finding is comparable to others. [6-8]

In this study 25%to 31% patients were smokers, compared to 80% in the world report of OSCC patients by Neville. Only one patient used alcohol, very low compared to world rates, due to religious influence. [6-8]

Table I- Age distribution of patients with OSCC in blood and tissue groups

Age	Blood		Tissue	
	Frequency	Percent	Frequency	Percent
37-46	6	20.7	4	25
47-56	5	13.3	3	18.75
57-66	4	13.8	3	18.75
67-76	7	24.1	3	18.75
77-86	7	24.1	3	18.75

Table II- Pair t test in chromosomal disorders in blood and tissue patients with oral SCC

Disorder	T- value	P-value
Double minutes	1.46	0.16
Fragment	2.79	0.14
Breakage	4.49	0.0001
Aneuploidy	3.50	0.003
Polyploidy	2.23	0.014
Dicentric	0.62	0.544

Since pair t-test between fragment, breakage, aneuploidy, polyploidy are greater than t-value (t=2.13) (df=15), there is significant difference between tissue and blood in chromosomal disorders with 95% assurance.

It should be noted that in Western countries, heavy drinkers are usually heavy smokers, and these two factors reinforce each other in incidence of OSCC. This combination is rare in Iran.

In this study almost all of patients (93.1%) were over 40 years old, which is consistent with previous reports (Table I).[6-8] Also all patients showed chromosomal disorders which were both structural and numerical. This is consistent with Hermsen's study, which analyzed 11 patients with OSCC, and observed numerical chromosomal disorders in all of them [11], and contrasts with the study by Ravindran et al which found only 12 chromosomal disorders in 75 OSCC patients, all of numerical type. [12]

The numerical disorders included polyploidy and aneuploidy and structural disorders included fracture, dicentric, fragment and double minute.

No previous study had reported dicentric and double minute cases; our study found 6 patients with dicentric and 4 with double minute. Dicentric and double minute disorders were observed only in patients who showed severe chromosomal disorders. There may be a relationship between these two disorders and recurrence. The cause and process of this phenomenon are unknown, but the malignant cell may contain an amplified area that has many transcriptions from proto-oncogenes. Indeed, in the carcinogenesis process, any chromosomal or cytogenic disorders activate proto-oncogenes, or inactivate suppress tumor suppressor genes.

Patel et al studied chromosomal disorders in 8 patients with OSCC (using cell colony method). In that study all patients showed chromosomal disorders. Chromosomes 1, 7, 8, 9, 11 and x exhibit some disorders. They related this to the locus of proto- oncogenes, and tumor suppressor genes to the area of fracture, and suggested that these phenomena may have a role in carcinogenesis. [13]

This study showed that disease process in OSCC patients is directly related to

chromosomal disorders. The patients, who were in recurring stage, had severe defects and disturbances. The disorders didn't confine to one chromosome but many chromosomes were involved.

In our study four patients showed severe chromosomal disorders. Most of them (three patients) showed recurrence and were under radiotherapy. This is consistent with studies by Owens and Patridge. [14,15]

Owens studied the patients with OSCC. Five cases that also previous treatment (4 radiotherapy one surgery) showed chromosomal disorders. [14]

Patridge et al. studied 48 patients with OSCC, and observed that chromosomal disorders increased as tumor recurrence increased, and patient survival decreased. [15]

In that study 4 patients who showed severe chromosomal disorders were all men and over 75 years of age. This may indicate a correlation between age and chromosomal disturbances that may relate to decrease of immuno-surveillance in old people.

We have previously noted that severe chromosomal disorders were observed in patients with recurrence, and who had undergone radiotherapy. Any connection between radiotherapy and chromosomal disorders and recurrence should be subject to further research.

It may be useful, to evaluate chromosomal disorders in blood samples of patients with OSCC. It is essential to find out whether patients with OSCC will have any change in chromosomal disorders after surgery, chemotherapy or radiotherapy or not. Therefore it is a question that can we find out recurrence after with obtaining numerous bloods sampling in certain periods?

As table II shows, for evaluating chromosomal disorders, tissue samples are better than blood samples. Therefore we suggest evaluating tissue samples for histopathology and chromosomal disorders to obtain more data. That perhaps helps to judge better about prognosis and survival.

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