

## **Chromosome Abnormalities of Oral Squamous Cell Carcinoma and Correlation to the Tumor Size and TNM Staging**

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### **Abstract**

It is well established that cancer is a multistage process involving a number of aberrant molecular events culminating in malignant transformation. Indeed the great majority of human cancers exhibit quite visible chromosome changes and the consistent chromosome abnormalities are essential for the malignant phenotype, the aim of the present study designed to assess the chromosomal aberration and the possible correlation with tumor size and TNM staging (T tumor size, N lymph node involvement, M tumor metastasis) of oral squamous cell carcinoma . We assessed the chromosomal aberration of sixteen patients with oral squamous cell carcinoma were studied, fresh and solid tumor was taken for mincing and transferred to a new tissue culture media, chromosomes staining was carried out with trypsin-Geimsa banding .

The results of the present studies showed Aneuploidy, loss of chromosomes, deletion of chromosomal 7 Ch7q (22 → 36), translocation t (7:4q) (22 → 36 :: 35) and dicentric chromosome and double minutes. Tumor size (T<sub>3</sub> + T<sub>4</sub> + T<sub>X</sub>) only represented a positive findings. TNM staging only stage (III+IV) represented a positive chromosomal aberration.

In conclusion there was no specific chromosome or chromosomal aberration associated with oral squamous cell carcinoma. The tumor size and TNM staging system can be considered as a good clinical predictors for treatment plane and prognosis .

Keyword : chromosomal abnormalities, oral squamous cell carcinoma, tumor size TNM staging.

### **Introduction**

The etiology of oral cancer is not well understood at the present time <sup>(1,2)</sup>. Several etiological factors are recognized including tobacco, betel nut leaf quid, alcohol and nutritional state <sup>(3)</sup>. Other suggested risk factors include immunodeficiency's <sup>(4)</sup>, or may be due to various DNA viruses and Candida Infection <sup>(5)</sup>. There is an increasing evidence that human papilloma virus and herpes simplex virus play a role in the etiology of oral cancer <sup>(6,7,8)</sup>, Other believed that streptococcus anginosus which exist in the mouth as a normal flora located mainly in the gingival and dental plaque was implicated in the carcinogenesis of head and neck squamous cell carcinoma <sup>(9)</sup>. Other predisposing factors such as poor oral hygiene, ill fitting dentures, sharp teeth and chronically infected gingival may cause persistent irritation of oral carcinoma mucosa. Mechanical irritation can act as promoter but not as an initiator and dental factor may have a similar role in oral carcinoma in human <sup>(10)</sup>. Polycyclic aromatic hydrocarbons represented the most potent chemical carcinogens, and a great majority of chemical carcinogens are mutagens either directly or following enzymatic activation <sup>(11)</sup>. The etiology of oral squamous cell carcinoma may be related to the use of depleted uranium <sup>(12)</sup>. There is a strong suggestion that depleted uranium might associated with development of oral squamous cell carcinoma .

It is now well established that cancer is a complex genetic disease <sup>(13)</sup>. The great majority of human exhibit visible chromosomal changes <sup>(14)</sup>. The type of chromosome aberration either showed deletion of part or an entire chromosome, or translocation, or gain of chromosomal material by amplification of an oncogene <sup>(15)</sup>. The chromosomal aberration in oral or head and neck squamous cell carcinoma are demonstrated in chromosome 3,9,11,17. <sup>(16)</sup> Losses of chromosomal regions at 9p, 17p does correlates strongly to tobacco and alcohol in the etiology of head and neck tumors <sup>(17)</sup>. The consistent chromosome abnormalities are essential for the malignant phenotype <sup>(18)</sup>. The clinical behavior of head and neck squamous cell carcinoma is difficult to predict based on classical histopathological parameters alone <sup>(19)</sup>. The present study was designed to

investigate the chromosomal aberrations associated with oral squamous cell carcinoma and its correlation with the size of tumors and TNM staging system .

Materials and methods :

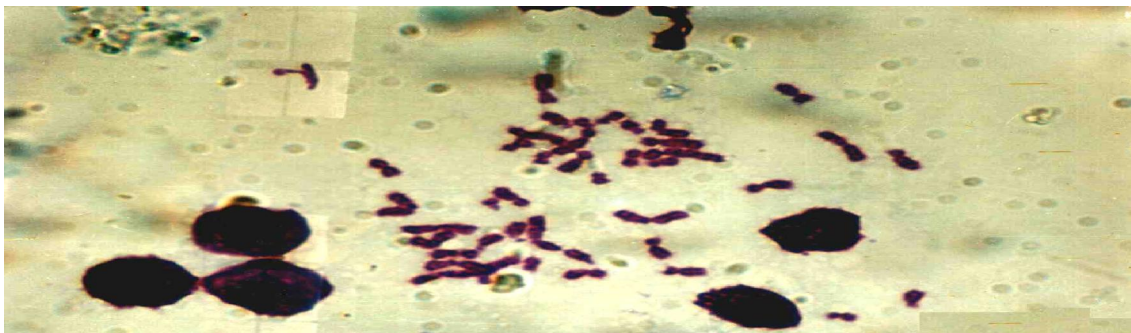
Sixteen patients were selected from plastic and reconstructive center ,(Facio-Maxillary unit ), the cytogenetic investigation was carried out in the central teaching – genetic laboratory of Baghdad medical center. Patients were selected who were diagnosed as oral squamous cell carcinoma. All tumor were graded according to the UICC TNM criteria.

The preparation of chromosome was carried out by a method cited by Sharma Archana et al 1983<sup>(20)</sup> . Fresh biopsy was taken and divided aseptically into two portion, one fixed in 10 % of buffered formalin for histopathological evaluation while other portion immersed in a tissue culture media 199 for cytogenetic study using an air flow cabinet . Fresh biopsy was taken and solid tumor was mincing for at least 30 minutes into fine pieces then transferred to a new test tube containing fresh tissue culture media which was supplemented with fetal calf serum 16 volume (mI), L – glutamine , penicillin 10000 IU /ml with streptomycin cited by DE Rooney et al 1998 <sup>(21)</sup> . Culture was incubated for 48h at 37C<sup>o</sup>, colchicine was added at a final concentration of 0.01 mg /lcc, then test tube was centrifuged for 10 minutes at 1800 rotation per minute . The supernatant fluid is discarded and the precipitate treated with 0.0752 kcl and kept in water bath at 37C<sup>o</sup> for 20 minute then centrifuged , the precipitate fixed in methanol : glacial acetic acid (3:1).

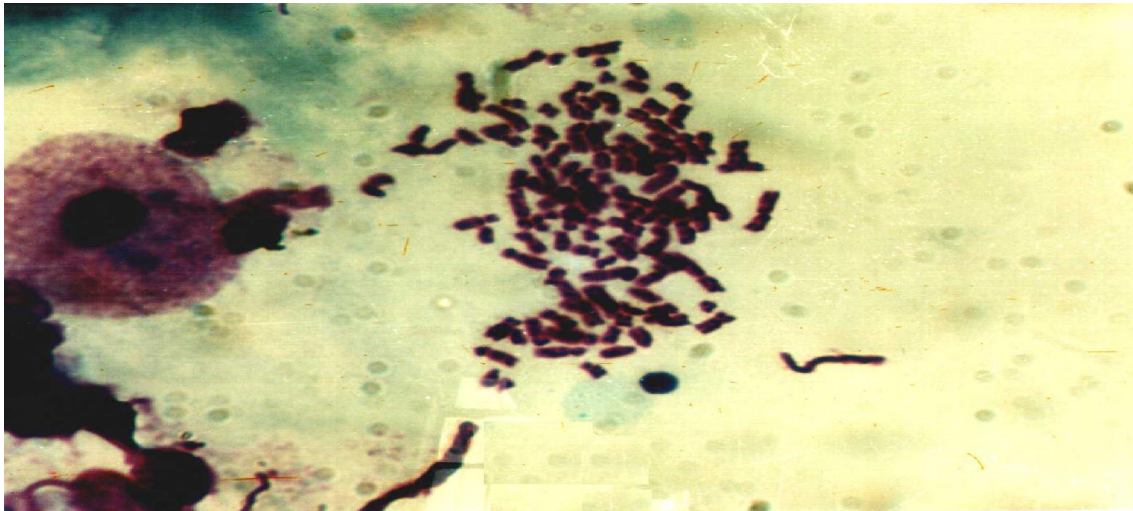
After that test tube was centrifuged The precipitate was dropped on cold damped slides from a height of 30 cm. The slides were dried in a stream of cold air ,and finally, chromosome staining was carried out with trypsin –Geimsa banding method . Trypsin concentration 0.25% at (25-28 )C<sup>o</sup>, phosphate buffed saline solution (PH7.2) ,Giemsa stain concentration of (2 gram /100cc) for 4-5 minutes, Garr buffer (PH 6.8).

### **Resul**

Sixteen patients were included age ranged from 19-80 years and the mean (41.5), 9 male (56.2%) and 7 female (42.8%). The male to female ratio was 1.28:1. Six specimens successfully cultured for cytogenetic findings. The findings showed aneuploidy hypoploidy Fig (1) and hyperdiploidy Fig (2) and was shared cytogenetic findings in all 6 cases. In one case there was hypoploidy and hyperdiploidy, and loss of chromosomes included all chromosomes, chromosomes 22 was lost in all 3 cases of position numerical cytogenetic findings while chromosomes x, 2, 3, 4, 7, 8, 10, 11, 12, 13, 14, 17, 20, 21 were lost in 2 cases, and chromosomes y, 1, 6, 15, 16, 18, 19 were lost in one case (table 1)

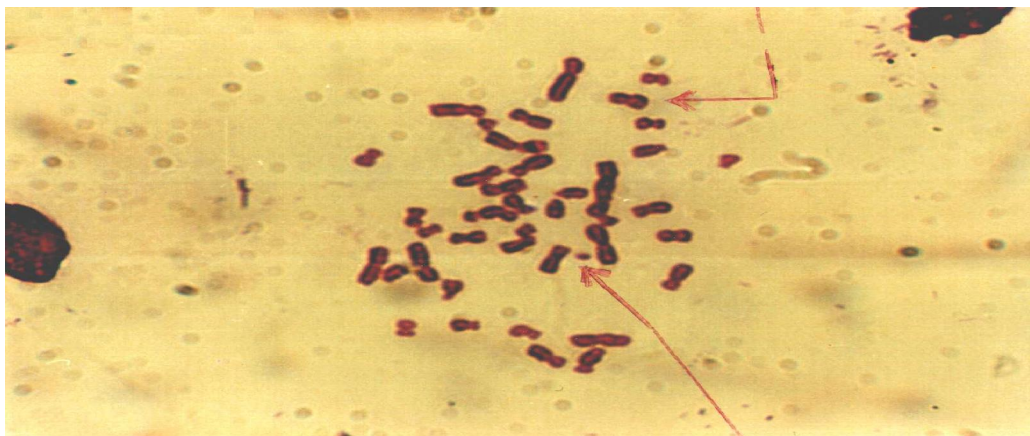


**Fig. (1) Hypoploidy (aneuploidy) Trypsin- Giemsa (X400)**



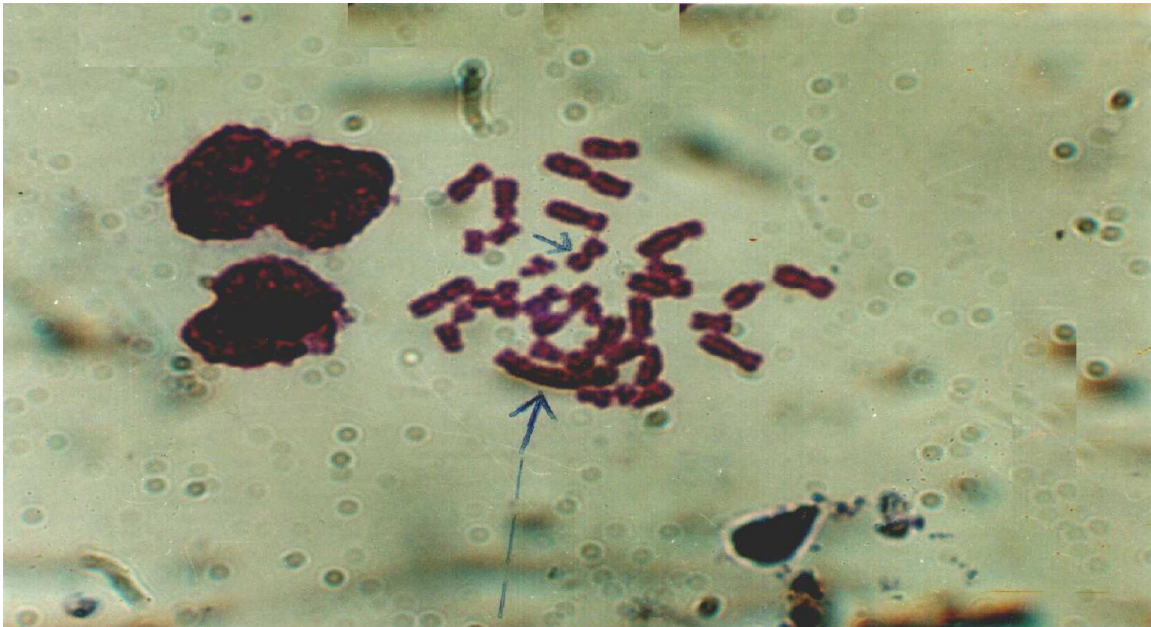
**Fig. (2) Hyperploidy (aneuploidy) Trypsin- Giemsa (X400)**

**Deletion of 7(q22 : : 36) →**  
Chromated fragment

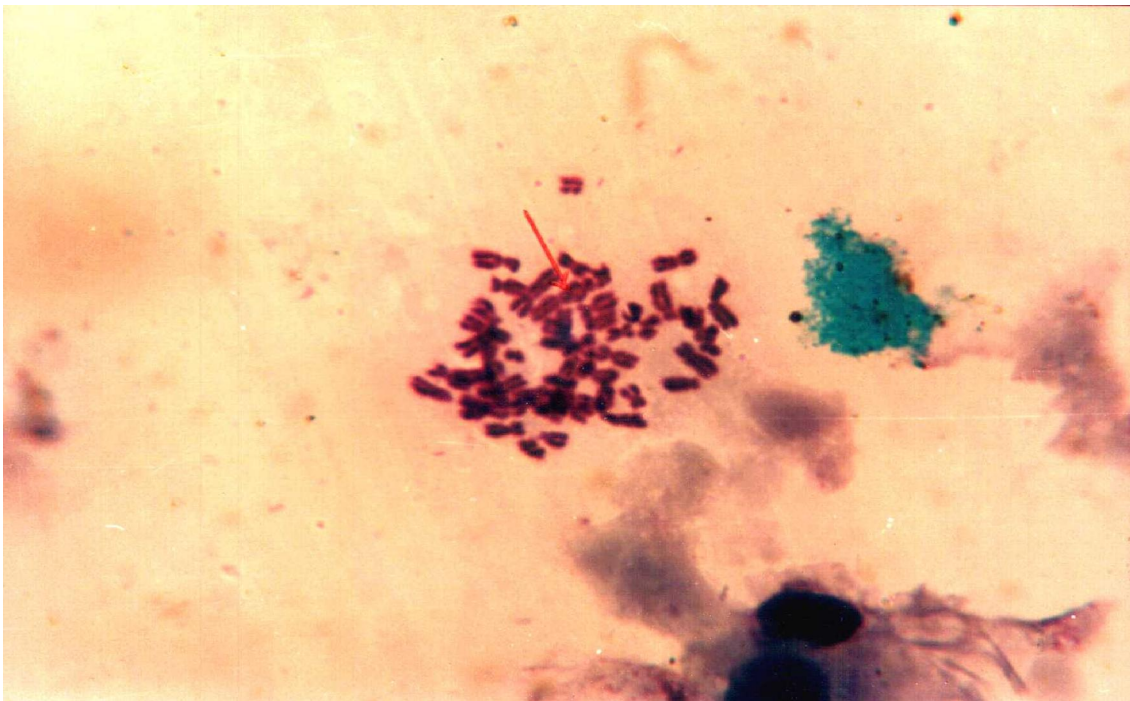


**Fig. (3)Chromosomes culture of oral squamous cell carcinoma showing deletion of 7(q22 : : 36) and chromated fragment, trypsin-Giemsa (X400). 5**





**Fig(4) Chromosomes culture of oral squamous cell carcinoma showing translocation of chromosome translocation t (7:4)(q22 36::q35)trypsin Giemsa(X400).**



**Fig(5) Chromosomes culture of oral squamous cell carcinoma showing dicentric of chromosome, trypsin-Giemsa(X400).**

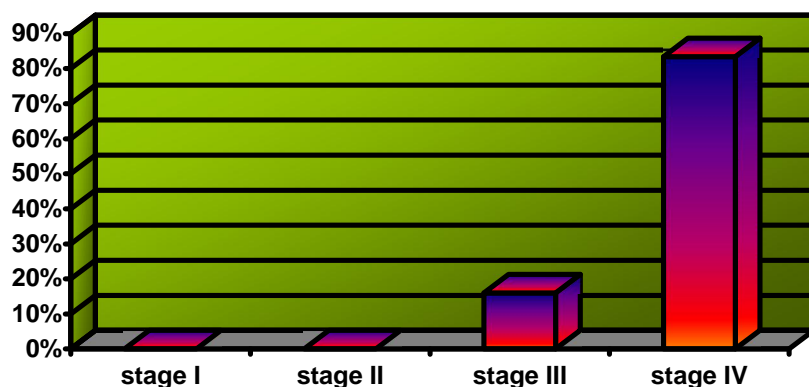
Case No.	Numerical cytogenetic findings	Structural cytogenetic findings
1.	A. Aneuploidy (Hypoploidy) B. Loss of chromosomes 2,3,4,13,14,17,19,20,21,22	A. Double minutes B. Deletion of ch 7q (22 ::
2.	A. Aneuploidy (Hypoploidy) B. Loss of chromosomes 2,3,4,5,7,8,10,11,12,13,14,15,16,17,18,20,21,22,x	A. Double minutes B. translocation (7,4q)(22 36 :: q 35)
3.	Aneuploidy (Hyperdiploidy)	
4.	Aneuploidy (Hyperdiploidy)	
5.	Aneuploidy (Hyperdiploidy)	
6.	A. Aneuploidy (Hyperdiploidy) (Hypoploidy) B. Loss of chromosomes 1,6,7,8,9,10,11,12,22,x,y	Dicentric chromosome

**Result**

shows, the early tumor size Tis (carcinoma in situ), T<sub>1</sub> [ tumor size 2cm or less ], and T<sub>2</sub> [tumor size more than 2cm but not more than 4cm in greatest dimension ] did not show any positive cytogenetic findings while T<sub>3</sub> [tumor more than 4 cm] and T<sub>4</sub> [tumor invades adjacent structures] and Tx [ tumor size can not be assessed, very large ] showed positive cytogenetic findings (100%) table 2 . Advanced malignant squamous cell carcinoma of the oral cavity such as T<sub>3</sub> , T<sub>4</sub> showed a positive cytogenetic findings.

**Table (2): Relation between tumor size and positive cytogenetic findings of oral squamous carcinoma**

Tumour size	Positive cytogenetic finding No.	(%)
Tis + T1+ T2	0	(0.0%)
T3 +T4+Tx	6	(100%)
Total	6	(100%)



**Fig(6) Histogram showing TNM staging of oral squamous cell carcinoma by positive cytogenetic findings. 7**

**Table ( 3 ) Clinical and histopathological parameters with cytogenetic findings of oral squamous carcinoma .**

	Gender	Age	Clinical feature	Site of lesion	T.N.M System	Stage	Histopathology	Degree of Differentiation.	Chromosomal findings Numerical structural	
1	F	54y	Exophytic	Check	T <sub>3</sub> N <sub>0</sub> M <sub>0</sub>	II1	Adeno cystis car.	Poorly diff.	Aneuploidy Monosomy Double minute like G	Delete d of Ch 7q (22 --- --- 36 )
2	M	41y	Endophytic	Lower alveolar gingiva	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	II	Sq.cell.car.	Moderately diff .		
3	M	42y	Endophytic	Upper alveolar gingiva Check	T <sub>2</sub> N <sub>1</sub> M <sub>0</sub>	III	Sq.cell.car.	Well diff .		
4	F	71y	Endophytic	Upper & lower alveolar gingiva Check	T <sub>4</sub> N <sub>2</sub> M <sub>x</sub>	IV	Sq.cell.car.	Poorlydiff .		
5	F	45y	Enophytic	Upper & lower alveolar gingiva Check	T <sub>4</sub> N <sub>2</sub> M <sub>x0</sub>	IV	Sq.cell.car.	Moderately diff .	Aneuploidy Monosomy Double minute like G	Transl ocaion Ch 4:7 T(7 ,4q)(22 ----36) (35)
6	F	43y	Endophytic	lower alveolar gingival lateral border of tongue	T <sub>4</sub> N <sub>2</sub> M <sub>x</sub>	IV	Sq.cell.car.	Moderately diff .		
7	F	19Y	Endophytic	Upper & lower lip check	T <sub>4</sub> N <sub>2</sub> M <sub>x</sub>	IV	Sq.cell.car.	Moderately diff .	Hyperdiploidy Aneuploidy	
8	M	58y	Endophytic	lower alveolar gingival check retro molar area	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	II	Sq.cell.car.	Moderately diff		

9	M	31y	Exophytic	Hard palate upper alveolar	T <sub>4</sub> N <sub>1</sub> M <sub>0</sub>	IV	Adeno cystis car.	Poorly diff .	Hyperdiploidy Aneuploidy chromosomes 92	
10	F	51y	Exophytic	Hard palate	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	II	Adeno carcinoma	Well diff.		
11	F	37y	Endophytic & Exophytic	Check lateral border of tongue and floor	T <sub>4</sub> N <sub>2</sub> M <sub>0</sub>	IV	Sq.cell.car.	Moderately diff	H Aneuploidy yperdiploidy	
12	M	63y	Endophytic	Upper lip check and hard palate	T <sub>3</sub> N <sub>0</sub> M <sub>0</sub>	III	Sq.cell.car.	Moderately diff		
13	M	68y	Endophytic	Check and upper alveolar gingiva	T <sub>4</sub> N <sub>2</sub> M <sub>0</sub>	IV	Sq.cell.car.	Moderately diff.		
14	M	58y	Endophytic	Upper and lower lip	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	II	Sq.cell.car.	Well diff .		
15	M	80	Exophytic	Floor of mouth	T <sub>x</sub> N <sub>3</sub> M <sub>x</sub>	IV	Sq.cell.car.	poorly diff .	Aneuploidy Monosomy	Dicentric
16	M	51y	Exophytic	Lateral border of tongue	T <sub>2</sub> N <sub>1</sub> M <sub>0</sub>	III	Adeno cystis car.	Moderately diff.		



**Discussion :**

The goal for prognostic factor assessment has shifted to the identification of chromosomal aberration in patient with oral squamous cell carcinoma. Analysis of the presented study have shown that males are more affected than females with ratio of 1.28:1, and the incidence of male to female with oral squamous cell carcinoma had been lowered , due to increased cigarette smoking among women <sup>(22)</sup> beside women working in factories who might subjected to pollution and stress .

The result of successful cultures of our cases of squamous cell carcinoma was ( 37.5% ),but once we exclude cases received chemotherapy and or radiation (genotoxic therapy) , the percentage of successfulness increased to (54.5% ) The explanation of that, due to many factors include weather temperature , time consumed for transporting solid tumors from theater to the cytogenetic laboratory, and the technical difficulties encountered in solid tumors cytogenetic in general and in analysis of squamous cell carcinoma in particular <sup>(17,23)</sup>

Aneuploidy hypoploidy Fig.(1) and /or hyperdiploidy Fig. (2) are a significant chromosomal abnormalities observed, hypoploidy it self may lead to inactivation of tumour suppressor genes which might lead to disturb the cell cycle . The majority of human tumours manifest an imbalance of the regulatory cell cycle control processes <sup>(19)</sup> while hyperploidy may lead to unbalance between proto -oncogene and tumor suppressor gene . Molecular studies of squamous cell carcinoma of the head and neck have demonstrated multiple genetic abnormalities such as activation of various oncogenes, tumor suppressor gene inactivation <sup>(24)</sup> . Aneuploidy it self was a diagnostic feature of oral squamous cell carcinoma and designates an abnormal number of chromosomes missing and may result in incomplete genomic DNA and more likely loss of critical important genes. Extra chromosomes my lead to unbalanced gene expression <sup>(25)</sup> and chromosomal abnormalities were responsible for transformation to malignant cells . Analyzing these tumors was yielded to a significant clinical cytogenetic correlation been of great diagnostic and prognostic importance <sup>(17)</sup> ,and chromosomal changes are essential for the malignant phenotype <sup>(18)</sup> Loss of chromosomes table (1) either (autosomal and / or sex linked ) may lead to inactivation of critical tumor suppressor gene , gain or loss of chromosome may be a consequence of dis-junction and loss of chromosome was considered as a diagnostic cytogenetic features of oral squamous cell carcinoma .A great majority of human cancer is a complex genetic disease <sup>(13)</sup>

Loss of chromosomes 3,5,8,9,18 and 21 have been commonly identified with oral squamous cell carcinoma<sup>(26)</sup>,while chromosomal changes in oral or head and neck squamous cell carcinoma are detected in chromosome 3,9,11 and 17 <sup>(16)</sup>

Deletion of an entire chromosome might inactivate the tumor suppressor genes<sup>(27)</sup>,and the result demonstrate deletion of 7(q 22 → 36)Fig.(3).

Loss of chromosome segment carrying the wild allele form cell heterozygous for a tumor suppressor gene leads to the absence of its tumor suppressor product<sup>(18)</sup>. Deletion of ch ((q22 → 36) is the most commonly associated with myelodysplastic syndrome<sup>(17)</sup> .

The presence of translocation t (7:4)(q 22 → 36::q35) Fig.(4). In our cases and it is significant as might activate an oncogene or in activate a tumor suppressor gene<sup>(17)</sup> . Translocation it self lead to increase cell cycle<sup>(18)</sup> . With accumulation of mutations that activate proto-oncogenes and in activate tumor suppressor genes which end with loss of normal control

of proliferation<sup>(29)</sup>. Cell proliferation is a central and key phenotypic expression in all type of malignant neoplasia<sup>(30)</sup> ,

Double minute Table (1) is a cytogenetic feature point to amplification of oncogenes and oncogenes lead to continuous cell division . Amplification it self (reduplication) of genes can be detected by molecular studies or identified cytogenetically in the form of double minutes<sup>(27)</sup>. Double minutes have been observed in chromosomes of many primary tumors and reflect the amplification of the MYC gene, which is greatly associated with environmental factors<sup>18)</sup>.In our country the numerical and structural chromosomal aberration of oral squamous cell carcinoma might be due to, malnutrition, smoking and consumption of alcohol, immune suppression, recurrent periodontal diseases, sever pollution, use of chemical carcinogens, poverty and life style.

Dicentric phenomena Fig.(5) which is a translocation between two chromosomes , it is quite possible for the two fragments of the chromosome to fuse with centromeres, leaving the two a centric fragment to fuse into a large a centric fragment. Dicentric chromosomes are rare phenomena, and the most common dicentric chromosome observed predominantly in male phenotype <sup>(28)</sup>.And also may occur in tumors as a result of translocation<sup>(14)</sup>.

The relation between tumor size and positive cytogenetic findings, in our cases with early squamous cell carcinoma such as carcinoma in situ (Tis) and tumour with (T1 and T2) did not showed any positive cytogenetic findings table (2), our explanation of negative cytogenetic findings might be due to the , slight changes that could not be detected by the method been used in this study which require, molecular investigation, or the carcinoma of the head and neck arise as result of accumulation of 6 to 10 genetic events<sup>(31)</sup>. Kinzler and Vogel –stein were reported that at least seven mutations have been considered to be necessary for the development of malignancy<sup>(32)</sup>. The only certain way of avoiding cancer is not to be born, to live ,and in-cure the risk.

The relation between TNM staging system and positive cytogenetic findings showed one case with stage III represent ( 16.6%) of total six cases, and five cases with stage IV which constitute (83.4%). It was

noticed in our cases, that stage IV of oral cancer showed a high chromosomal aberration accumulation in comparison with stage III. All five positive cytogenetic findings of stage IV revealed that T4 but not all N score was with a lymph node involvement ( palpable).

### **conclusion**

It seem there was no specific chromosome and chromosomal aberration associated with oral carcinoma. Aneuploidy (hypoploidy,hyperploidy) and deletion of chromosome fragment 7q(22 36) are the most common changes associated with malignancy while the dicentric of chromosomes were rare . The translocation of t (7:4)(q22 36 : : 35) was not reported in the literature. The positive cytogenetic findings could be considered as a diagnostic aid in addition to histopathology, CT scan, MRI, tumor markers and other recent modalities used in oral carcinoma for diagnosis and detection.

The tumor size and TNM staging system were a good clinical predictors for treatment and prognosis of oral squamous cell carcinoma.

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