

# Immunohistochemical evaluation of FHIT and WWOX expression in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma

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## ABSTRACT

**Background:** Oral squamous cell carcinoma is the most prevalent malignant neoplasm of the oral cavity which results from accumulated genetic and epigenetic alterations. It is not always inexorable and may be reversible if early intervention in the process can occur to prevent further genetic mutation and disease progression. The FHIT gene is a tumor suppressor gene located in FRA3B region which is the most active common fragile site, where DNA damage leading to aberrant transcripts and translocations frequently occur. The WWOX is a tumor suppressor gene that plays a central role in tumor suppression through transcriptional repression and apoptosis, with its apoptotic function the more prominent of the two. This study aimed to evaluate and compare the immunohistochemical expression of FHIT and WWOX in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma and to correlate the expression of the mentioned markers with the clinicopathological features and to show the expression of studied markers with each other.

**Materials and methods:** Fifty formalin-fixed, paraffin embedded tissue blocks (10 cases of normal oral mucosa, 19 cases of oral epithelial dysplasia, and 21 cases of oral squamous cell carcinoma) were included in this study. Immunohistochemical staining was performed using anti FHIT polyclonal antibody, and anti WWOX polyclonal antibody.

**Results:** Positive IHC of FHIT was detected with high score in all cases of NOM, 16 cases (84%) of OED and 18 cases (86%) of OSCC. For WWOX expression positive IHC detected with high score in all cases (100%) of NOM, 14 cases (74%) of OED and 15 cases (71%) of OSCC. There was statistically highly significant correlation of both markers in OED and non significant correlation in OSCC, with significant differences among studied groups.

**Conclusions:** These results signifying both markers cooperative tumor suppressive role and potential pathological transition from normal oral mucosa to dysplastic epithelium and subsequently cause malignant oral lesions.

**Keywords:** NOM, OED, OSCC, FHIT, WWOX. (J Bagh Coll Dentistry 2014; 26(4):102-107).

## الخلاصة

**الخلفية:** سرطان الفم الحرشفي هو أكثر أنواع الأورام الخبيثة حدوثاً في التجويف الفمي ينتج من تراكم التغيرات الوراثية والتغيرات المتعاقبة العارضة. هذا التحول ليس بالعملية الصعبة حيث يمكن استرجاعه اذا تم التدخل مبكراً لمنع الطفرات الوراثية المتكررة ومنع تقدم المرض. ان جين (FHIT) هو جين مثبط للورم ويقع في منطقة (FRA3B) التي تعتبر أكثر منطقة في الجين معروفة بالهشاشة والتي يحصل فيها اصابة الحامض النووي المؤدية الى استئساخ وتبدل مواقع جينية متكررة الحدوث. ان جين (WWOX) هو جين مثبط للورم ويلعب دوراً مركزياً في اخماد الورم عن طريق تفعيل عملية النسخ الجيني وتنظيم عملية موت الخلايا المنظم والتي تعتبر الطريقة الاهم لتنشيط الورم.

**اهداف الدراسة:** تقييم ومقارنة الاظهار المناعي النسيجي الكيميائي لـ (FHIT) و (WWOX) في الغشاء الفموي الطبيعي والحثل النموي الطلاني الفموي وسرطان الفم الحرشفي. وربط الاظهار الخاص بهذه المؤشرات الحياتية مع الصفات السريرية المرضية وكذلك الاظهار الخاص بالمؤشرات الحياتية المدروسة مع بعضها الاخر.

**المواد وطرق العمل:** شملت هذه الدراسة خمسين قالباً نسيجياً مثبتاً بالفورمالين ومطوراً في شمع البارافين (10 قوالب للغشاء الفموي الطبيعي، 19 قالباً للحثل النموي الطلاني الفموي وكذلك 21 قالباً لسرطان الخلايا الحرشفية). تم صبغ المقاطع بصبغة المناعة النسيجية الكيميائية باستخدام المضادات الحيوية لـ (FHIT) وكذلك (WWOX).

**النتائج:** ان الاظهار المناعي الصبغي للـ (FHIT) كان ايجابياً وتصنيف عال في جميع حالات الغشاء الفموي الطبيعي (100%)، وفي (16) حالة (84%) للحثل النموي الطلاني الفموي و(18) حالة (86%) لسرطان الحرشفي الفموي. بينما كان الاظهار المناعي الصبغي للـ (WWOX) ايجابياً والتصنيف عالياً في جميع حالات الغشاء الفموي الطبيعي و(14) حالة (74%) من الحثل النموي الطلاني الفموي و(15) حالة (71%) من سرطان الفموي الحرشفي كانت هنالك علاقة معنوية عالية احصائياً لكلا المؤشرين الحيويين في الحثل النموي الطلاني الفموي وعلاقة غير معنوية في سرطان الخلايا الحرشفية الفموي. مع اختلافات معنوية لهما بين المجاميع المدروسة.

**الاستنتاجات:** النتائج المذكورة لكلا المؤشرين الحيويين دلت على دورهما المميز والمتلازم في تثبيط التحول الورمي من غشاء فموي طبيعي الى حثل نموي طلاني وبالنتيجة يؤدي الى اورام فموية خبيثة.

## INTRODUCTION

The most common oral cancer of epithelial origin is oral squamous cell carcinoma (OSCC), corresponding to almost 95% of all lesions and to about 38% of malignant tumors of the head and neck <sup>(1)</sup>.

It remains a lethal disease in over 50% of the cases diagnosed annually, due mostly to late detection of advanced stage cancer <sup>(2)</sup>.

The transition from normal oral epithelium to oral dysplasia and cancer results from accumulated genetic and epigenetic alterations <sup>(3)</sup>.

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The best-known precursor lesion is epithelial dysplasia, which is histologically detectable and often presents clinically as white or red mucosal patches called leukoplakia and erythroplakia <sup>(4)</sup>.

Oral squamous carcinogenesis is a multistep process in which multiple genetic events occur that alter the normal functions of oncogenes and tumor suppressor genes. This can result in increased production of growth factors or numbers of cell surface receptors, enhanced intracellular messenger signalling, and/or increased production of transcription factors.

In combination with the loss of tumor suppressor activity, this leads to a cell phenotype capable of increased cell proliferation, with loss

of cell cohesion, and the ability to infiltrate local tissue and spread to distant sites <sup>(5)</sup>.

Common fragile sites are chromosome regions which observed in metaphase chromosomes. Those genes in these regions are more susceptible to breakage, rearrangements and deletions than other genes and played an important role in the carcinogenesis <sup>(6-7)</sup>.

The fragile histidine triad (FHIT) and the WW-domain oxidoreductase gene (WWOX) are tumor suppressor genes that encompass the FRA3B and FRA16D fragile sites at chromosomes 3p14.2 and 16q23.3, respectively <sup>(8)</sup>.

Fragile histidine triad protein is known to play a role in the process of neoplastic transformation. It has been demonstrated that FHIT gene inactivation is manifested by a lack or very low concentration of Fhit protein in tissues collected from tumours in many organs, including head, neck, breast, lungs, stomach or large intestine <sup>(9)</sup>.

It was indicated that loss of FHIT protein not only correlated with tumour aggressiveness but also was detected in pre-cancerous lesions, pointing out the possible importance of the FHIT gene in the initiation of cancer <sup>(10)</sup>.

WWdomain-containing oxidoreductase is an enzyme that in humans is encoded by the WWOX gene. WW domain-containing proteins are found in all eukaryotes and play an important role in the regulation of a wide variety of cellular functions such as protein degradation, transcription, and RNA splicing. This gene encodes a protein which contains 2 WW domains and a short-chain dehydrogenase/reductase domain (SRD) <sup>(11)</sup>.

The nature of the various proteins that the WWOX protein can interact with, such as c-Jun, TNF, p53, p73, AP-2gamma, and E2F-1, suggests that WWOX plays a central role in tumor suppression through transcriptional repression and apoptosis, with its apoptotic function the more prominent of the two <sup>(12)</sup>.

Poor prognosis or unfavorable clinical outcome in patients is associated with low or absent expression of WW domain-containing oxidoreductase (WWOX) protein in cancer specimens <sup>(13)</sup>.

This study aimed to evaluate and compare immunohistochemical expression of FHIT and WWOX in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma, correlate the expression of the mentioned markers with the clinicopathological features and to show the expression of studied markers with each other.

## MATERIALS AND METHODS

Fifty formalin-fixed, paraffin embedded tissue blocks (10 cases of normal oral mucosa, 19 cases of oral epithelial dysplasia, and 21 cases of oral squamous cell carcinoma) were included in this study dated from (1980 till 2013), were obtained from the archives of the department of Oral & Maxillofacial Pathology/ College of Dentistry/ University of Baghdad; Al-Shaheed Ghazi Hospital/ Medical City / Baghdad; and Al Kadhimiya teaching Hospital.

After histopathological reassessment of haematoxylin and eosin stained sections for each block, an immunohistochemical staining was performed using anti FHIT polyclonal antibody, and anti WWOX polyclonal antibody.

## RESULTS

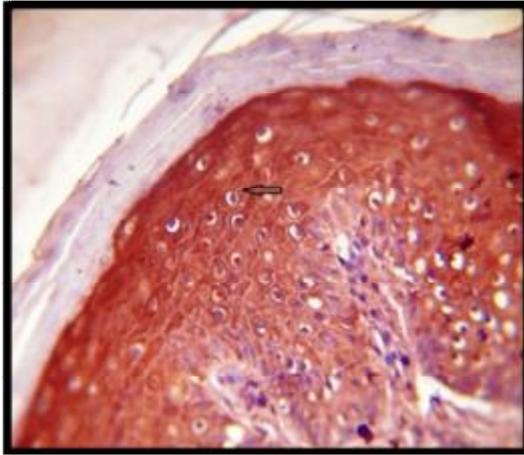
Positive FHIT Immunostaining was detected as brown cytoplasmic expression.

Diffuse Positive immunostaining of FHIT was detected with high score in all cases (100%) of NOM. For OED result showed that score IV (diffuse positive staining) was found in 16 cases (84.12%), while remaining cases distributed among score I (negative), II (sporadic positive staining) and III (focal positive staining) with one case (5.26%) for each score.

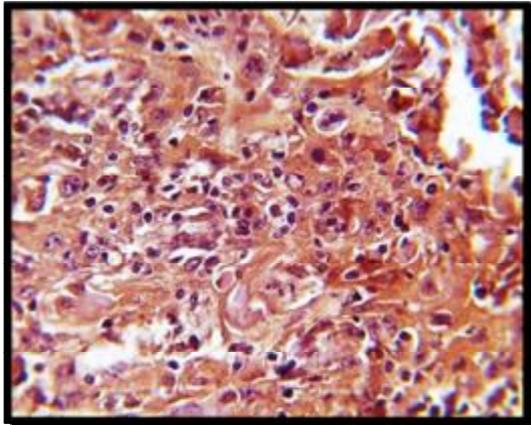
Concerning the degree of FHIT expression in OSCC, result showed that score IV was found in 18 cases (85.71%), followed by score I (negative) in 2 cases (9.52%) and one case (4.76%) with score II. fig (1,2,3).



**Figure 1: Positive cytoplasmic expression of FHIT in NOM(400x).**



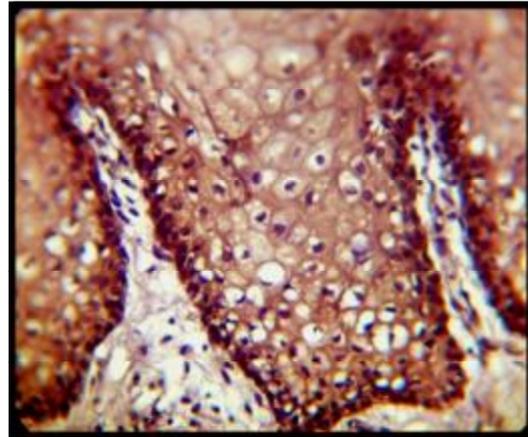
**Figure 2: Positive cytoplasmic expression of FHIT in mild OED (400x).**



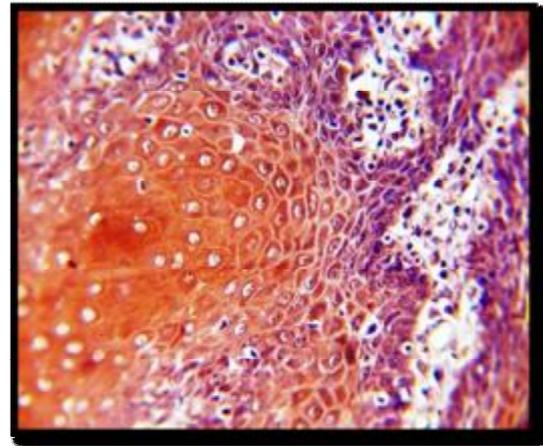
**Figure 3: Positive cytoplasmic expression of FHIT in moderate differentiated OSCC (400x).**

Positive WWOX Immunostaining was detected as brown cytoplasmic or cytoplasmic with nuclear expression. According to the degree of expression all cases (100%) of NOM showed score III (strong positive staining). For OED the result showed that Score I (negative) was found in 2 cases (10.53%), Score II (weak positive) 3 cases (15.79%) and Score III (strong positive) 14 cases (73.68%).

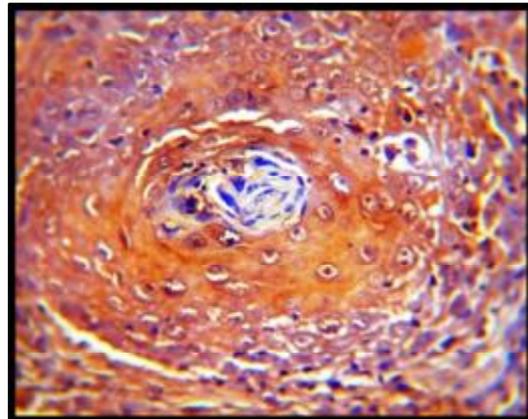
Concerning degree of expression of WWOX in OSCC, result revealed that majority of cases were score III 15 cases (71.43%) followed by score I and II both found in 3 cases (14.29%). fig (4,5,6).



**Figure 4: Positive cytoplasmic expression of WWOX in NOM (400x).**



**Figure 5: Positive cytoplasmic and nuclear expression of WWOX in severe OED (400x).**



**Figure 6: Positive cytoplasmic and nuclear expression of WWOX in well differentiated OSCC (400x).**

Regarding the correlation between both markers and clinicopathological parameters of oral epithelial dysplasia and oral squamous cell carcinoma there was non significant correlation except for the correlation between WWOX and clinical presentation of oral epithelial dysplasia which was statistically significant.

**The correlation between FHIT and WWOX expression**

In OED cases, the results of the present study revealed statistically a **highly significant** correlation between the two studied markers (p-value= 0.000) according to Spearman's Correlation Table 1. In OSCC cases, the results revealed statistically a **non significant** correlation between the two markers (p-value= 0.59). Table 2

**Table 1: Correlation between FHIT and WWOX markers in OED**

Markers		WWOX
FHIT	r	0.86
	p-value	0.000 (HS)*

**Table 2: Correlation between FHIT and WWOX markers in OSCC**

Markers		WWOX
FHIT	r	0.12
	p-value	0.59 (NS)

**Differences of FHIT and WWOX markers between each two groups**

Regarding the correlation of FHIT and WWOX between each two groups and according to Mann-Whitney U test, the results of this study revealed statistically **highly significant** difference between NOM and OSCC for FHIT expression (p-value=0.002), while for WWOX expression results showed **highly significant** difference between NOM and OED and between NOM with OSCC (p-value=0.000). **Non-significant** difference was found between OED and OSCC for both markers. Table 3

**Table 3: Mann-Whitney U test between each two groups**

Markers	Groups		Mann-Whitney U test	P-value
FHIT	NOM	OED	53.5	0.054 (NS)
		SCC	34.5	0.002 (HS)
	OED	SCC	176.5	0.529 (NS)
		SCC	13	0.000 (HS)
WWOX	NOM	OED	13	0.000 (HS)
		SCC	9.5	0.000 (HS)
	OED	SCC	184.5	0.683 (NS)

**DISCUSSION**

**Clinicopathological findings of OED**

Regarding the epidemiological parameters, including age, gender, site, and clinical presentation, studies showed variable results; in the present study most of OED cases were in patients over 40 years of age, such finding showed that the incidence of dysplastic changes increase with age, and this finding is in agreement with the finding of previous studies (14,15).

Regarding clinical presentation of OED, white lesion (leukoplakia) represent the most predominant clinical presentation (47.37%), which came in accordance with the findings of (14, 15).

**Clinicopathological findings of OSCC:**

Concerning site distribution of OSCC, the tongue represented the most predominant site in this study, this is in agreement with the results of (15, 16) and disagree with (17, 18).

Intraoral squamous cell carcinoma predominates in the ventrolateral tongue, floor of mouth and mandibular alveolus regions. These areas have been postulated to form a 'gutter zone' into which soluble carcinogens may pool and exert their neoplastic influence (19).

Concerning clinical presentation the most predominant presentation recorded in this study was a mass presented in 12 cases (57.14 %), followed by ulcerative lesion 8 cases (38.10%).

**Assessment of FHIT immunohistochemistry**

As we expected, all cases of NOM (100%) showed score IV (diffuse positive staining), this finding was in agreement with previous studies by (20-21) who showed strong positive immunoreactivity for FHIT in normal oral epithelium.

The FHIT gene is expressed at low levels in most tissues of the body but interestingly the highest expression was detected in epithelial cells and tissues. Fragile histidine triad gene is inactivated in epithelial tumors and specifically those exposed to environmental carcinogens. This loss of expression seems to occur early in the development of these cancers while, in other cancers, it may be a later event corresponding to progression and aggressiveness (22).

Concerning OED cases, the results of this study showed that score IV (diffuse positive staining) of FHIT was observed in 16 cases (84%), and one case (5%) for each negative (score I), score II and score III. Regarding correlation between FHIT and clinicopathological features, there was statistically non significant correlation of FHIT with age, gender, site and clinical presentation. It

was indicated that loss of Fhit protein not only correlated with tumor aggressiveness but also was detected in pre-cancerous lesions, pointing out the possible importance of the FHIT gene in the initiation of cancer<sup>(10)</sup>.

In oral squamous cell carcinoma, the results of this study showed that the highest score of FHIT was observed in 18/21 cases (85%), negative or low expression was found in 15%, this come in agreement with the data of<sup>(20)</sup>.

Previous studies showed a range of 4% to 68% absent or markedly reduced FHIT expression in OSCC<sup>(23, 25)</sup>.

This discrepancy may depend on the specificity and sensitivity related to different FHIT antibodies, staining protocol, scoring system, and tissue collection<sup>(21)</sup>.

#### Assessment of WWOX immunohistochemistry

In NOM, all cases (100%) showed score III (strong positive staining), agreed with the result of<sup>(26)</sup> by using RT-PCR.

Concerning OED cases, the results of this study showed that score III of WWOX was observed in 14 cases (73.68%), reduced expression was found in 5 cases (26.32%).

Regarding correlation between WWOX and clinicopathological features, there was statistically non significant correlation of WWOX with age, gender and site, while there was significant correlation with the clinical presentation of OED (white lesion), which was the most clinical presentation in this study.

Concerning OSCC cases the results of this study showed that score III of WWOX was observed in 15 cases from 21 with loss of expression in about 30%, this finding come in agreement with<sup>(26)</sup>.

Many studies have also shown that WWOX protein expression is reduced or lost in tumor cells compared with normal cells and that finding could be associated with certain clinical or pathological parameters<sup>(27, 28)</sup>.

#### Correlation between FHIT and WWOX IHC expression in OED and OSCC:

Fragile histidine triad gene exhibits many features similar to WWOX. Both of them are most active fragile genes and they are large genes with the length of more than 1 Mb. Both genes are located in common fragile sites and lie in a region of homozygous deletions and present a high frequency of aberrant RT-PCR products in tumors<sup>(29)</sup>.

The result of the present study showed a high expression rate of both markers in NOM, with parallel reduction of the expression in OED and

OSCC. It was difficult to find previous studies correlate FHIT and WWOX immunohistochemical expression in OED and OSCC, however we compared our results with other close results. Regarding the correlation between FHIT and WWOX expression in each group, the result revealed a statistically highly significant correlation in OED and non significant correlation in OSCC.

In accordance with previous reports, lost or reduced FHIT and WWOX expression has been shown to be an important step in the initiation of tumorigenesis in a variety of tumors, including breast, lung, esophagus, kidney, cervix and other organs<sup>(30, 31)</sup>.

#### Studied groups' comparison between FHIT and WWOX

The result of the present study showed highly significant difference between NOM and OSCC concerning FHIT expression, For WWOX expression, results showed highly significant difference between NOM and OED and between NOM and OSCC.

In nasopharyngeal carcinoma (NPC) study by<sup>(32)</sup> showed that significant difference was found in FHIT and WWOX mRNA expression levels between (NPC) tissues and non cancerous tissues, and FHIT mRNA expression was strongly correlated with WWOX (NPC) patient.

These results support the concept that both markers are tumor suppressor and reduce in premalignant or malignant lesions.

Therefore, we speculate that statistically significant difference between FHIT and WWOX among groups of NOM, OED and OSCC cases observed in this study suggest their close and synergistic, cooperation and coactivation in the malignant potential of oral lesion and can be use as early diagnostic markers for evaluating these lesions.

Finally, FHIT and WWOX could be use as target for gene therapy in patients with malignant oral lesions through its tumor suppressive role.

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