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Assessment of the Role of Insulin Growth Factor Receptor in Angiogenesis, Metastasis, and Prognosis in Oral Squamous Cell Carcinoma by Immunohistochemical study and Real-time PCR

Ibrahim Mohamed Abd Elwahab[1], Amr Helmy Mustafa El Bolok [2], Enas Alaa Eldin Abd Elaziz [3], Maii Ibrahim Sholqamy[4]

[1] PhD Candidate of Oral and Maxillofacial Pathology, Faculty of Dentistry, Minia University, Cairo, Egypt.
[2] Professor of Oral and Maxillofacial Pathology, Faculty of Dentistry, Minia University, Cairo, Egypt.
[3] Assistant Professor and Head of Oral and Maxillofacial Pathology, Faculty of Dentistry, Minia University, Cairo, Egypt.
[4] Assistant Professor of Oral and Maxillofacial Pathology, Faculty of Dentistry, Minia University, Cairo, Egypt

Corresponding Author: ebrahimdahab88@gmail.com
amrelbolok@gmail.com, Maii.ali@mu.edu.eg

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ABSTRACT

Objective: *In this study, we will investigate IGFR1 expression among OSCC samples suggests a correlation with tumor differentiation. Also, the variability in CD34 expression across different OSCC samples.*

Methods: *Both quantitative and qualitative analysis can be done with immunohistochemistry for IGFR1 and CD34 and real-time PCR. the data collected are Statistically analyzed with IBM® SPSS® (ver. 26. SPSS Inc., IBM Corporation, Armonk, NY, USA).*

Results: *In this study, immunohistochemical and real-time PCR results show that IGFR1 expression is highest in severely differentiated OSCC samples. Poorly differentiated tumors are likely to show higher IGFR1 expression. Increased CD34 levels are associated with higher tumor grades and more aggressive behavior.*

Conclusion: *This suggests that IGFR1 is known to promote tumor growth by activating various signaling pathways. The present outcomes from this study are going in same way with many documentations. These reports have indicated the role of IGFR1 in angiogenesis, metastasis, and prognosis of OSCC.*

KEYWORDS: *OSCC, PCR, Insulin Growth Factor, IGFR1.*

1 | INTRODUCTION

Head and neck squamous cell carcinomas (HNSCC) are among the most aggressive tumors, with oral squamous cell carcinoma (OSCC) representing the vast majority, it accounts for 90% of all oral cancers. One of the commonest forms of cancer is head and neck cancer. Lip, oral cavity, and oropharynx combined were

responsible for about 4,47,751 new cancer cases with an estimated 2,28,389 deaths in 2018, which accounts for 2.4% of all cancer deaths. (1,2).

Early detection of cancer is a key factor for improved prognosis and increased patient survival rate. Even though the oral cavity can be easily examined and assessed by direct visual inspection, most OSCC cases are not identified early. This most likely ensues because patients do not seek dental care on a regular basis and most oral cancers in the early stages are asymptomatic. Moreover, dentists may not be aware of the different clinical presentations of OSCC and misdiagnose cancers as reactive or benign lesions. (3-5).

Despite the new diagnostic modalities in oral cancer detection, biopsy and histopathologic analysis remain the gold standard to diagnose OSCC. (6).

The insulin-like growth factor (IGF)/insulin family of growth factors is an evolutionally conserved system which plays a crucial role in the growth and development of many tissues and the regulation of overall growth and metabolism. (7).

Insulin-like growth factors 1 and 2 are single-chain polypeptides with a high sequence homology to pro-insulin. The half-lives, transportation, and bioavailability of the IGFs circulating at high concentrations in the bloodstream and extracellular fluids are modulated by several high affinity IGF-binding proteins (IGFBP1–6). (8).

In the past decades, a large body of evidence has arisen, supporting a key role for IGF-1R signaling in various types of human cancers. A number of studies performed in the last two decades have demonstrated a role for this receptor in the transformation of cells, cancer cell proliferation, as well as in metastatic events (9).

In oral cancer, the quantification of the number of genetic copies through real-time PCR using either DNA or RNA in studies on gene expression has been reported for human tumors, including breast cancer, follicular lymphoma, stomach cancer, prostate cancer and Ewing's sarcoma. In carcinoma of the head and neck, this new technology has been mainly applied for the detection of Epstein-Barr virus in nasopharynx cancer and squamous cell carcinoma in lymph nodes (10-11).

CD34 can serve as an important prognostic marker in OSCC, with higher expression levels indicating tumors that are more likely to metastasize and result in poorer patient outcomes. Assessing CD34 expression levels in OSCC could help identify patients at higher risk and guide therapeutic decisions, such as anti-angiogenic therapies that target the tumor's blood supply. (12-14).

The aim of this study is to measure the Microvascular density of different grades of Oral Squamous Cell Carcinoma, correlate the expression of Insulin Growth Factor Receptor in Oral Squamous Cell Carcinoma with Microvascular density and compare the expression of Insulin Growth Factor Receptor in Oral Squamous Cell Carcinoma with Real-time PCR.

2 | Material and Methods

I- MATERIAL:

A-Tissue specimens:

Fifteen archival paraffin embedded specimens were collected from the archives of the Oral and Maxillofacial Pathology Department, Minia University, of cases diagnosed with different grades of oral epithelial dysplasia, **low risk** (mild) and **high risk** (moderate, severe) according to classification of WHO and squamous cell carcinoma (well-differentiated, moderately-differentiated and poorly-differentiated). (15)

Six archival paraffin embedded specimens including normal oral mucosa were collected from cases diagnosed with lesions other than dysplasia and carcinoma (e.g mucous retention and extravastion cysts), and were used as control.

Five microns thick sections were cut and submitted for routine hematoxylin and eosin staining to be examined under light microscope for confirmation of the previous diagnosis.

B. Immunohistochemical Reagents:

1- Primary Antibody:

- Mouse monoclonal IgG1 antihuman CD34 Antibody will purchased from ***Santa Cruz Biotechnology, USA***
- Mouse monoclonal IgG1 Anti-IGF-1 Receptor /IGF1R Antibody will purchased from ***Santa Cruz Biotechnology, USA***

2- Universal kit:

The Ready-to-Use LAB-SA immunodetection kit from Invitrogen (Cat# 85-9043) was used for immunostaining.

II-METHODS

A-Histopathological examination:

A section from each specimen was stained by routine haematoxylin and eosin (H&E) to confirm diagnosis under ordinary light microscope.

B-Immunohistochemical protocol:

Immunohistochemistry is a technique for identifying cellular or tissue constituent (antigens) by means of antigen-antibody reaction. The site of antibody binding is identified either by direct labelling of antibody, or by use of a secondary labelling method. The amino acid side-chains of the variable domain of an antibody form a cavity which is geometrically and chemically complementary to a single type of antigen epitope. The analogy of a lock (antibody) and key (antigen) and precise fit required explains the high degree of antibody-antigen specifity seen. The associated antibody and antigen are held together by a combination of hydrogen bonds, electrostatic forces, and Vander Waals forces (16).

The evaluation of immune-staining was performed by pathologists who was blind for experiment details, the immune-positive cells were counted in each region of interest (ROI) using a counting grid and their proportion among the total counterstained cell population. The stained areas of the ROI were digitally marked and the percentage of stained areas was determined using a computer program.

The protein expression intensity calculated using the was immune-reactive score (IRS) for IHC-data interpretation. The immune-reactive score (IRS) gives a range of 0–12 as a product of multiplication between positive cells proportion score (0–4) and staining intensity score (0–3) (17).

Table (1): The immune-reactive score (IRS)

A (percentage of positive cells)	**B (intensity of staining)**	**IRS score (multiplication of A and B)**
0 = no positive cells	0 = no color reaction	0-1 = negative
1 = <10% of positive cells	1 = mild reaction	2-3 = mild
2 = 11-50% positive cells	2 = moderate reaction	4-8 = moderate
3 = 51-80% positive cells	3 = intense reaction	9-12 = strongly positive
4 = >80% positive cells	**Final IRS score (A × B): 0-12**	

C-Evaluation of the immunostaining

The photomicrographed fields will be analyzed using image analysis software (Image J, 1.27z, NIH, USA). MVD will calculated by selecting three microscopic fields of highest neovascularization (vascular hotspots) indicated by immunostainings with CD34 under low magnification. Individual microvessels will manually outlined using freehand draw option in the image analysis software.

For IGFR1, Immunohistochemical staining will be evaluated under a light microscope by Intensity and Area percentage. Photographic images will be captured using a digital camera. Five fields of view at x200 magnification will examine and cells will count in total per specimen. The intensity will evaluate as: negative (0), weak (1), moderate (2), strong (3).

D. Gene expression analysis using Real-time PCR

1. Total RNA extraction from Formalin fixed paraffin tissue (FFPT)

Total RNA was extracted from FFPT, using the RNeasy FFPE Kit, cat no: 73504 (Qiagen, Hilden, Germany)

2. Reverse Transcription

The reverse transcription step was performed by the QuantiTect Reverse Transcription Kit, cat. No: 205310, (Qiagen, Hilden, Germany). The reverse-transcription master mix was prepared on ice in total volume of 20µL, which was composed of 1µL of Quantitect Reverse transcriptase enzyme, 4 µL of RT buffer, 1 µL of RT primer mix, and 14 µL of genomic DNA, the reaction mix was mixed and then kept on ice. The reverse-transcription master mix contains all components required for first-strand cDNA synthesis except template RNA. The reaction mix was incubated for 15 min at 42°C, then it was incubated for 3 min at 95°C to inactivate Quantiscript Reverse Transcriptase. The reverse-transcription reactions were placed on ice and then real-time PCR was proceeded directly (18).

3. Insulin growth factor (IGF-1R) gene amplification analysis.

The IGF-1R gene was amplified from mRNA using QuantiTect primer assay QuantiTect Primer Assay [HS_IGF1R_2_SG, QuantiTect Primer Assay, Gene Globe ID: QT01153523 (cat no: 249900 (Qiagen, Germany) and the QuantiTect SYBR Green PCR Kit cat no: 204141 (Qiagen, Germany) and Hs_ACTB_1_SG QuantiTect Primer Assay (β-actin) cat no: 249900, ID: QT00095431 as housekeeper gene. All samples were analyzed using the 5 plex Rotor Gene PCR Analyzer (Qiagen, Germany).

3.4. Delta - Delta Ct calculation in qPCR

The Delta- Delta Ct method compares the difference of expression (ΔCt) between the gene of interest (IGF-1R) and the reference gene (β-actin) first in the experimental conditions, and separately in your positive controls.

ΔCt (sample) = Ct (gene of interest) – Ct (reference gene)

Then the method compares the difference between the experimental and the positive control samples.

ΔΔCt = ΔCt (experimental sample) – ΔCt (positive control)

We calculate the relative changes in gene expression between the two compared sequences by using the formula **2-ΔΔCt**, where 2 is the efficiency set at 100%.

Statistical analysis

Statistical analysis was performed with IBM® SPSS® (ver. 26. SPSS Inc., IBM Corporation, Armonk, NY, USA). Data explored for normality using Shapiro-Wilk test. Quantitative data were presented by mean and standard deviation, while qualitative data were presented as frequency and percentage. One way ANOVA test used to compare means between four groups with Bonferroni post hoc test for significant results. Pearson correlation analysis was used to describe the association between numerical variables within group. A statistically significant level was considered when p value < 0.05.

3 | RESULTS

The oral SCC samples were classified according to the histopathological grading system into well differentiated SCC (4 cases), moderately differentiated SCC (7 cases) and poorly differentiated SCC (4 cases).

1- Well differentiated squamous cell carcinoma

(A). Histopathological Results

All cases of well differentiated SCC showed features of epithelial dysplasia in the invading tumor cells. These include hyperchromatism, pleomorphism, abnormal mitoses, alterations in nuclear cytoplasmic ratio and Malignant cells exhibit relative uniformity in size and shape with prominent nucleoli. The invading neoplastic epithelial nests or sheets showed marked degree of keratinization in the form of epithelial pearls figure (1).

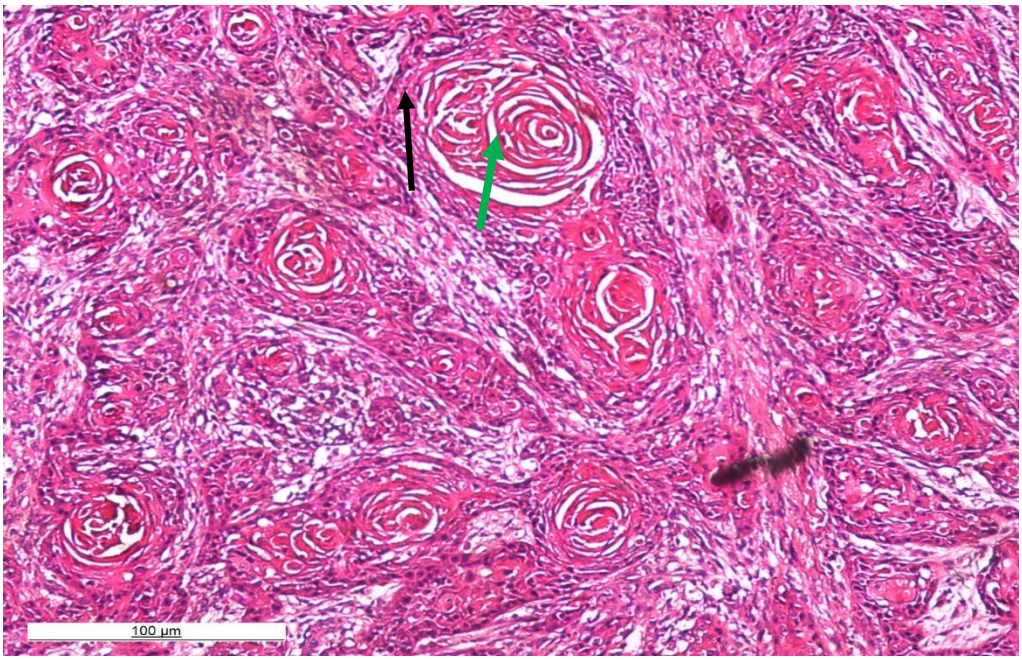

Figure (1): Photomicrograph of well differentiated OSCC showing invading tumor nests forming epithelial keratin pearls (green arrow), formed by surrounding neoplastic cells (black arrow) (H&E x100)

(B). Imunohistochemical Results of IGFR1

All cases showed IGFR1 immunoreactivity with cytoplasmic and nuclear localization. The invading tumor nests showed marked immunostaining of the entire cells either at the periphery or the center of the tumor sheets. Few malignant epithelial cells were immunonegative figure (2).

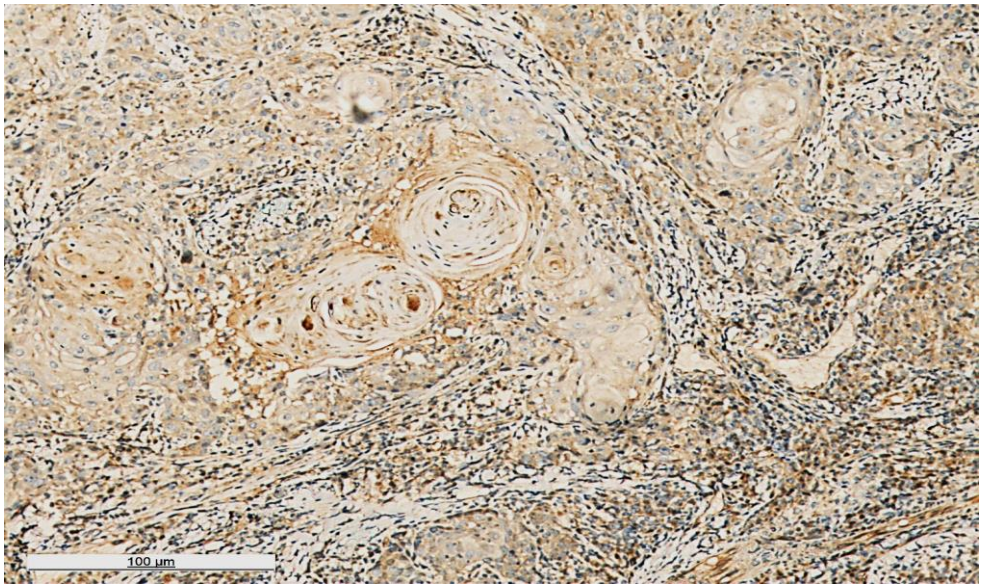

Figure (2): Immunohistochemical stained section of well differentiated OSCC with anti-IGFR1 show a membranous and cytoplasmic expression of IGFR1. Keratin pearls are also immunopositive (anti-IGFR1 x 100)

(c). Imunohistochemical Results of CD34

Immunohistochemical stained section of OSCC tumor tissue, stained with anti-CD34 showed few number of stained blood vessels in the stroma between the keratin and epithelial pearls figure (3).

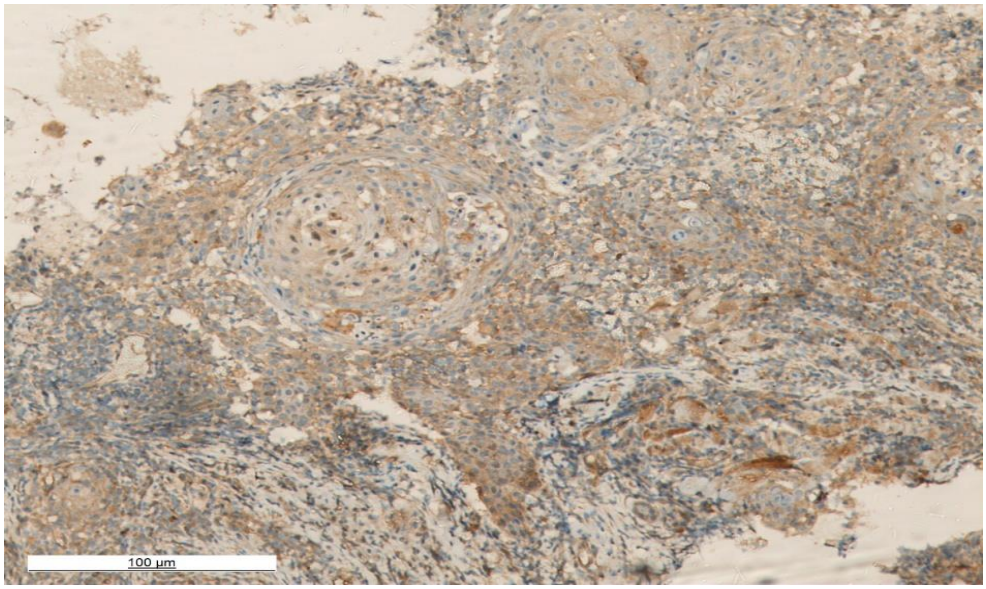

Figure (3): Immunohistochemical stained section of well differentiated OSCC with anti-CD34 showed few number of stained blood vessels in the stroma between the keratin and epithelial pearls (anti-CD34 x 100)

2- Moderately differentiated squamous cell carcinoma

(A). Histopathological Results

All cases of moderately differentiated SCC showed invasion of the connective tissue by neoplastic epithelial cells forming nests of variable sizes. Minimal degree of keratinization was noted in some nests figure (4).

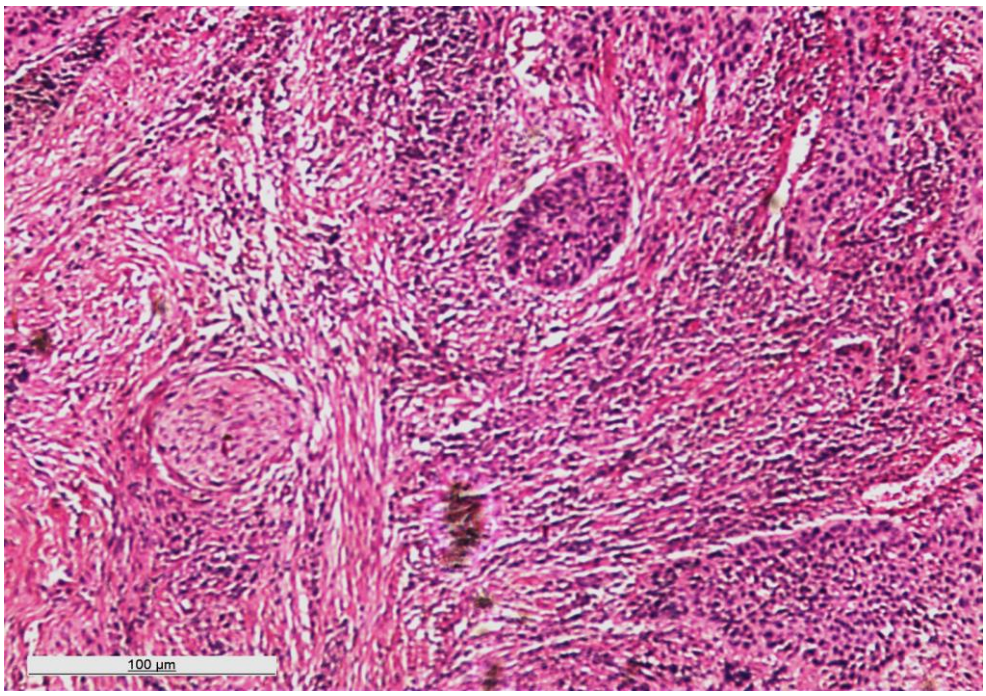

Figure (4): Photomicrograph of moderately differentiated OSCC showing invading tumor nests without epithelial keratin pearls formation (blue arrow) (H&E x100)

(B). Imunohistochemical Results of IGFR1

Immunoreactivity of IGFR1 showed cytoplasmic localization while one case showed cytomembranous localization IGFR1. The deeply invasive tumor nests showed marked immunostaining of central cells of the nests while the peripheral cells were immunonegative figure (5).

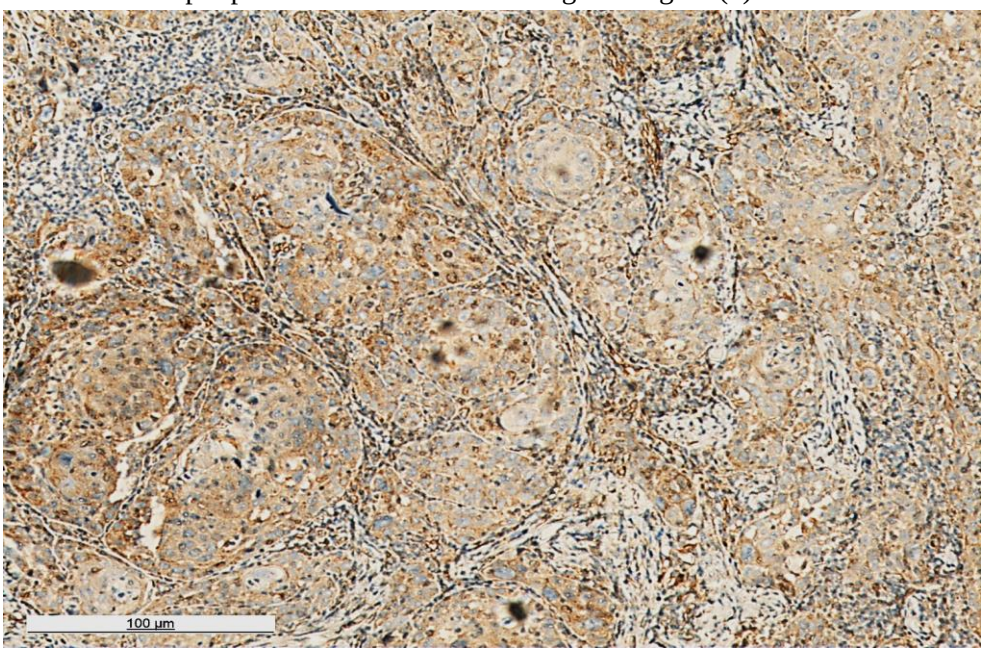

Figure (5): Immunohistochemical stained section of moderately differentiated OSCC with anti-IGFR1 show positive cytoplasmic immunostaining of all invading tumor nests (anti-IGFR1 x 100)

(c). Imunohistochemical Results of CD34

Immunohistochemical stained section of OSCC tumor tissue, stained with anti-CD34 showed increased number of small irregular stained blood vessels between cell nests figure (6).

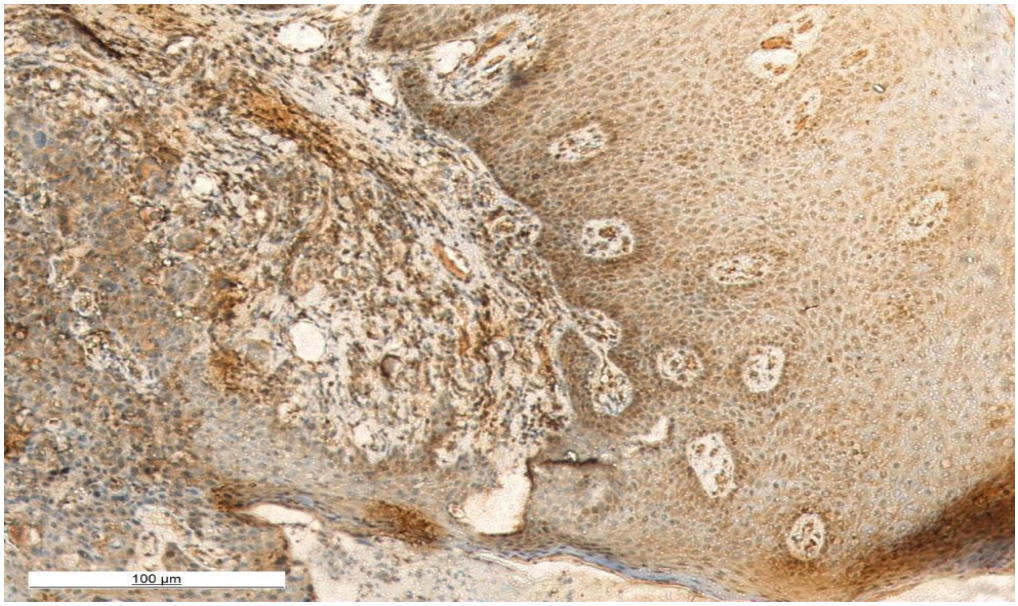

Figure (6): Immunohistochemical stained section of moderately differentiated OSCC with anti-CD34 showed increased number of small irregular stained blood vessels between cell nests (anti- CD34 x 100)

3- Poorly differentiated squamous cell carcinoma

(A). Histopathological Results

All sections of poorly differentiated squamous cell carcinoma showed scattered malignant epithelial cells with marked pleomorphism and hyperchromatism. These cells fail to form cell nests with absence of keratinization figure (7).

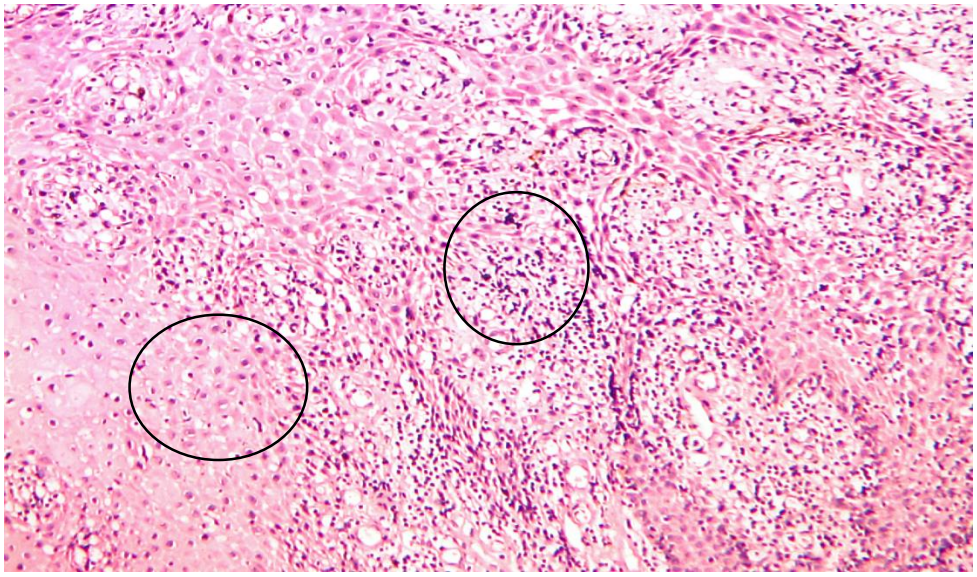

Figure (7): Photomicrograph of poorly differentiated OSCC showing showing invading malignant epithelial cells fail to form either cell nest or epithelial pearls. malignant epithelial cells with severe epithelial dysplasia

(black circles) (H&E x100)

(B). Imunohistochemical Results of IGFR1

All cases showed IGFR1 immunoreactivity with cytoplasmic localization. The number of immunopositive cells was increased in these undifferentiated cells. Some cells showed nuclear and cytoplasmic positive immunoreaction figure (8).

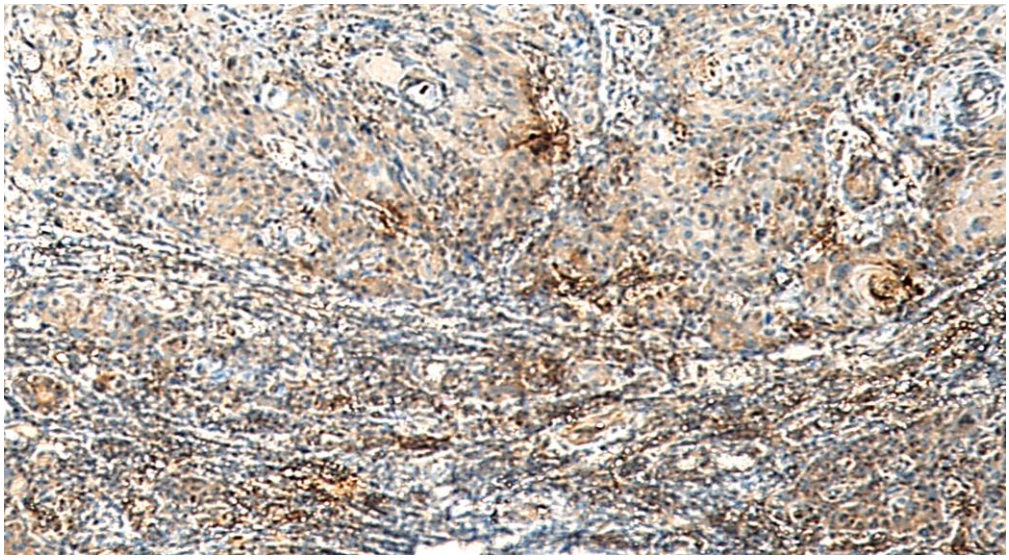

Figure (8): Immunohistochemical stained section of poorly differentiated OSCC with anti-IGFR1 show positive cytoplasmic immunostaining of malignant epithelial cells (anti-IGFR1 x 100)

(c). Imunohistochemical Results of CD34

Immunohistochemical stained section of OSCC tumor tissue, stained with anti-CD34 showed numerous number of small collapsed tortuous stained blood vessels between the tumor cells figure (9).

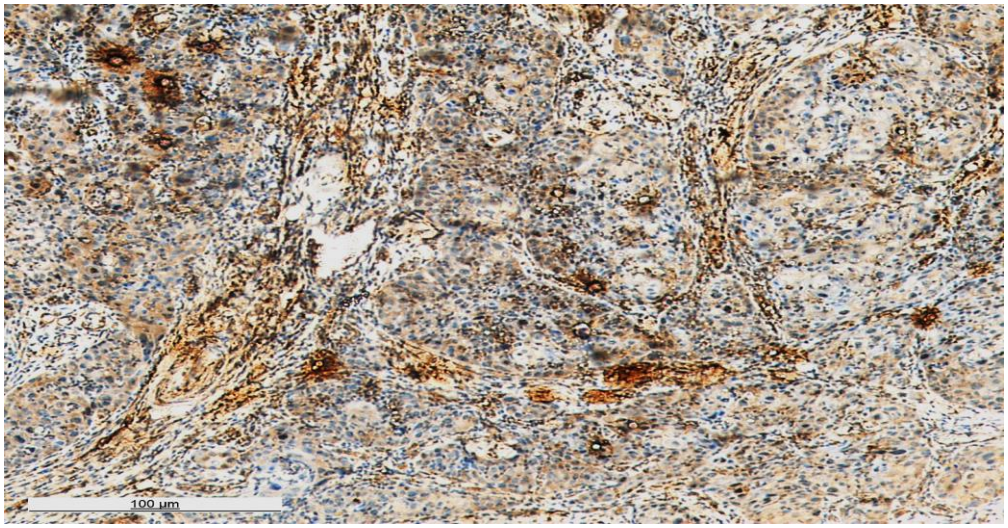

Figure (9): Immunohistochemical stained section of poorly differentiated OSCC with anti-CD34 show numerous number of small collapsed tortuous stained blood vessels, Invasion of the malignant epithelial cells into the surrounding structures. This includes the skeletal muscles, nerves and fat cells (anti- CD34 x 100)

Image Analysis Results

The immunohistochemical analysis of IGFR1 expression in Oral Squamous Cell Carcinoma (OSCC) and control samples revealed significant variations in IGFR1 levels, as represented by the Immunoreactive Score (IRS). The figure (10) shows differences in the percentage of IGFR1-positive cells and fluorescence intensity among the various OSCC samples and controls, providing insights into the potential role of IGFR1 in OSCC progression.

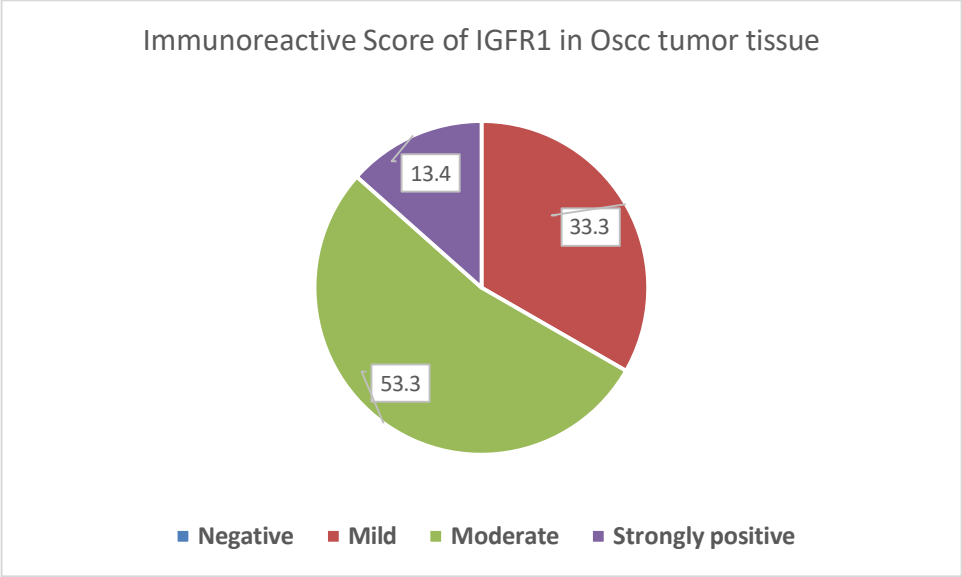

Figure (10): Immunoreactive Score of IGFR1 in OSCC tumor tissue

Several OSCC samples (e.g., Sample 1, Sample 2, and Sample 3) exhibit very high IRS values (12, 9, and 8, respectively), indicating a strong presence of IGFR1-positive cells (75% to 85%) and intense fluorescence staining. This suggests that IGFR1 is significantly upregulated in these tumors, which could correlate with higher tumor grade, increased cell proliferation, and aggressive behavior.

Some OSCC samples (e.g., Samples 7, 8, and 9) have moderate IRS values (6 to 8), indicating that while IGFR1 is upregulated compared to controls, the expression is less intense and the percentage of positive cells is lower. This moderate expression could correspond to moderately differentiated OSCC, where the tumor retains some features of normal differentiation.

The observed variability in IGFR1 expression among OSCC samples suggests a correlation with tumor differentiation. Poorly differentiated tumors are likely to show higher IGFR1 expression due to the receptor's role in promoting cell growth and resisting apoptosis, while well-differentiated tumors may show lower levels.

The immunohistochemical analysis of CD34 expression in OSCC tissue samples and control tissues highlights the variations in CD34 expression across different OSCC samples, providing insights into the tumor's angiogenic activity and aggressiveness.

The control samples (1, 2, and 3) exhibit low CD34 IRS values, ranging from 1 to 2, with minimal fluorescence intensity and a low percentage of positive cells (3% to 10%). This low expression is expected in healthy tissues, where angiogenesis is not actively occurring. The findings confirm that baseline levels of CD34 are minimal in non-cancerous tissues, reflecting a lack of increased microvascular density.

The moderate expression of CD34 may be indicative of tumors that are moderately differentiated, where the angiogenic response is active but not as pronounced as in more poorly differentiated and aggressive tumors.

The variability in CD34 expression across different OSCC samples suggests that increased CD34 levels are associated with higher tumor grades and more aggressive behavior. Tumors with high CD34 expression and higher IRS values are likely to exhibit a greater potential for metastasis and invasiveness due to the increased microvascular density.

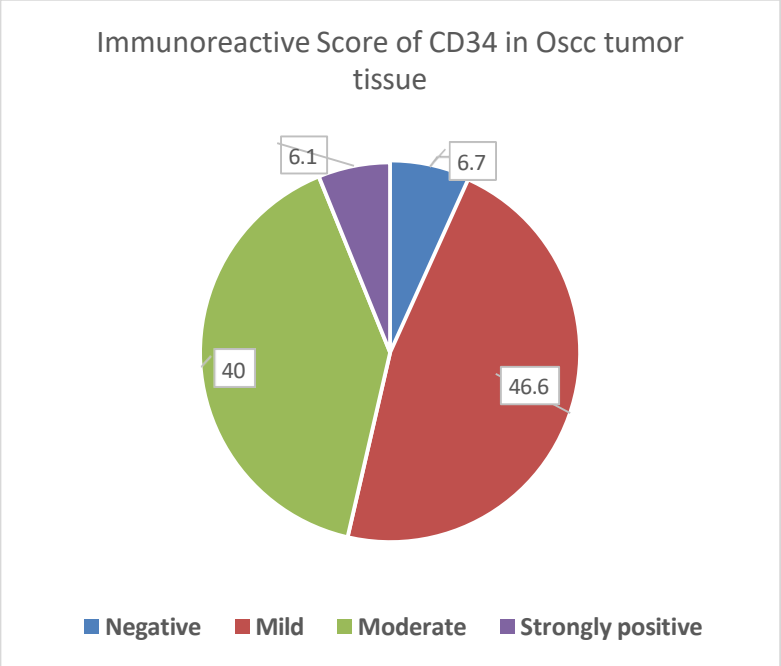

Figure (11): Immunoreactive Score of CD34 in Oscc tumor tissue

Correlating IGFR1 Expression with Microvascular Density in OSCC

IGFR1 is known to promote tumor growth and angiogenesis by activating various signaling pathways. The findings from immunohistochemical study of IGFR1 expression shows significantly upregulation in OSCC samples compared to control tissues. When we correlate these results with the findings of CD34 expression from, we observe:

- High IGFR1 Expression with High MVD: OSCC samples with high IGFR1 IRS values (e.g., Samples 1, 2, and 3) also show high CD34 IRS values, indicating a strong correlation between IGFR1 overexpression and increased microvascular density. This suggests that IGFR1 may be driving the angiogenic process in these tumors, leading to the formation of new blood vessels and supporting tumor growth.

- Moderate IGFR1 Expression with Moderate MVD: Similarly, samples with moderate IGFR1 expression also exhibit moderate CD34 expression, suggesting a proportional relationship between IGFR1 levels and MVD.

This correlation between IGFR1 expression and MVD supports that IGFR1 is not only involved in cell proliferation but also has a role in tumor angiogenesis and may be crucial.

Real-time PCR Results for IGFR1 Expression in OSCC

Table (2): Insulin growth factor 1 Receptor (IGF-1R) gene expression in OSCC

Serial	Sample code	CT [ACTB]	CT [IGFR1]	Δ CT [IGF-1R]	ΔΔ CT [IGF-1R]	FC [IGF-1R)
1	Sample 1	38.05	37.34	-0.71	-2.51	5.70
2	Sample 2	38.05	37.52	-0.53	-2.33	5.03
3	Sample 3	38.05	37.51	-0.54	-2.34	5.06
4	Sample 4	32.18	30.56	-1.62	-3.42	10.70
5	Sample 5	32.18	30.05	-2.13	-3.93	15.24
6	Sample 6	32.18	29.82	-2.36	-4.16	17.88
7	Sample 7	37.47	36.26	-1.21	-3.01	8.06
8	Sample 8	37.47	36.51	-0.96	-2.76	6.77
9	Sample 9	37.47	36.11	-1.36	-3.16	8.94
10	Sample 10	35.72	33.81	-1.91	-3.71	13.09
11	Sample 11	35.72	34.08	-1.64	-3.44	10.85
12	Sample 12	35.72	33.29	-2.43	-4.23	18.77
13	Sample 13	36.22	34.41	-1.81	-3.61	12.21
14	Sample 14	36.22	34.55	-1.67	-3.47	11.08
15	Sample 15	36.22	34.68	-1.54	-3.34	10.13
16	Control 1	36.81	38.26	1.45	-0.35	1.27
17	Control 2	36.81	39.15	2.34	0.54	0.69
18	Control 3	36.81	38.55	1.74	-0.06	1.04
19	Control 4	36.25	37.52	1.27	-0.52	1.44
20	Control 5	36.25	38.26	2.01	0.21	0.86

Mildly differentiated OSCC samples show an increase in IGFR1 expression with fold changes ranging from

5.03 to 8.06 compared to controls. This indicates that even in the early stages of tumor differentiation, IGFR1 is upregulated, suggesting its role in initiating tumor growth and progression.

The expression of IGFR1 is more pronounced in moderately differentiated OSCC samples, with fold changes ranging from 5.70 to 13.09. This upregulation suggests that IGFR1 plays a critical role in tumor proliferation and may contribute to the more aggressive behavior of moderately differentiated OSCC.

Severely differentiated OSCC samples exhibit the highest IGFR1 expression, with fold changes ranging from

12.21 to 18.77. The significant increase in IGFR1 expression in these samples indicates that this receptor is highly involved in the advanced stages of tumor progression, supporting its role in driving aggressive tumor growth and metastasis.

4 | DISCUSSION

Head and neck squamous cell carcinoma (HNSCC) is a significantcause of cancer worldwide. Incidence rates of these malignancies have been rising in most regions of the world. The tendency for local invasion and regional as well as distant metastases owing to the close proximity and uninhibited infiltration of local lymph nodes is high, and this is thought to be the greatest contributor

to the morbidity and mortality associated with OSCC (1- 3).

An adjunctive clinical approach consists of the use of biomarkers can offer further details during the clinical examination of a suspicious lesion to shorten the time of diagnosis and orient toward appropriate management in less time, with more accuracy and less invasivity (19).

This study was conducted to examine the evaluation of the role of Insulin Growth Factor Receptor in OSCC. The observed variability in IGFR1 expression among OSCC samples suggests a correlation with tumor differentiation. Poorly differentiated tumors are likely to show higher IGFR1 expression due to the receptor's role in promoting cell growth and resisting apoptosis, while well-differentiated tumors may show lower levels. CD34 can serve as an important prognostic marker in OSCC, with higher expression levels indicating tumors that are more likely to metastasize and result in poorer patient outcomes. Assessing CD34 expression levels in OSCC could help identify patients at higher risk and guide therapeutic decisions, such as anti-angiogenic therapies that target the tumor's blood supply (20).

The high expression of CD34 in these samples suggests that these tumors have developed an extensive network of new blood vessels to sustain rapid tumor growth and facilitate metastasis. This increased microvascular density is typically associated with more aggressive tumor behavior and poorer prognosis.

The Real-time PCR analysis reveals that Insulin Growth Factor Receptor 1 (IGFR1) expression is significantly upregulated in OSCC samples compared to control tissues. The fold change (FC) in IGFR1 expression varies across different grades of OSCC (mild, moderate, and severe differentiation), reflecting the heterogeneity of the tumors.

These results were found to be in line with **Ferreira et al., 2020** conducted a study focusing on how IGF-1 impacts multiple facets of tumor growth, including cell proliferation, migration, and angiogenesis in OSCC. The researchers illustrated that IGF-1 exerts its influence by activating key molecular pathways like PI3K-AKT and Hedgehog. These pathways are known to play crucial roles in enhancing the survival of tumor cells, facilitating their rapid growth, and increasing angiogenic processes that contribute to tumor expansion (21).

Our findings show a similar trend with **Jung et al., 2015** delves into the expression of various angiogenesis- related markers, including VEGF and IGFR1, across different grades of OSCC. They observed that these markers were significantly upregulated in poorly differentiated tumors, suggesting that their presence is associated with an aggressive and invasive tumor phenotype (22).

Also, in agreement with **Zhou et al., 2024** examined how extracellular vesicle-bound VEGF influences OSCC treatment resistance and tumor advancement. The findings indicate that the presence of VEGF within extracellular vesicles boosts the tumor's resistance to standard therapies and promotes more aggressive growth characteristics (23).

This aligns with our results here, as it demonstrates that elevated levels of growth factors such as IGFR1 and VEGF can modify the tumor environment, resulting in OSCC becoming more resilient to treatment. This further suggests that high IGFR1 levels may similarly contribute to a robust therapy-resistant tumor type, underscoring IGFR1's role in OSCC severity.

Chen et al., 2021 review supports our research by emphasizing the role of the tumor microenvironment and growth factor-mediated pathways in determining OSCC behavior and response to treatment. The inclusion of IGFR1 in the discussion reinforces its significance as a prognostic marker and potential therapeutic target, as suggested in our findings (24).

These results are in contrast to **Mountzios et al., 2013** who studied the prognostic significance of IGF1R expression in laryngeal squamous-cell carcinoma and its correlation with survival outcomes. While IGF1R protein expression was detected in LSCC, there was no significant association between IGF1R mRNA levels and patient survival outcomes. The researchers suggested that IGF1R mRNA might not be a reliable prognostic marker and that immunohistochemical detection of IGF1R protein levels is more informative in predicting clinical outcomes. The findings challenge our research emphasis on IGF1R mRNA levels as a reliable prognostic indicator for OSCC (25).

This study highlights the importance of the IGF-1/IGFR1 axis in tumor progression, not only in OSCC but also in other tumor types like schwannomas. The role of IGFR1 in enhancing tumor survival and growth via autocrine and paracrine loops supports our results and conclusions that IGFR1 plays a significant role in driving OSCC progression through similar mechanisms

Moreover, **Reinmuth et al., 2021** support our research by investigation how impairing the function of IGF-I receptor (IGF-IR) impacts the behavior of colon cancer cells, focusing on angiogenesis, tumor growth, and metastasis. The authors demonstrate that blocking IGF-IR significantly reduces the expression of VEGF, a key factor in angiogenesis, leading to a marked decrease in new blood vessel formation and smaller tumor sizes. The research also shows that the suppression of IGF-IR results in less aggressive tumor phenotypes, both in vitro and in animal models (26).

The findings of this study showing that IGF-IR is directly involved in regulating VEGF levels and angiogenesis, not only in colon cancer but also potentially in OSCC. The reduction in tumor growth and vascular development upon IGF-IR inhibition aligns with our results and conclusion that targeting IGFR1 could be a promising strategy to limit OSCC progression and metastatic potential.

However, these results were in contrast with **D. M. Ferreira et al., 2017** who explored the role of the PI3K/AKT/mTOR signaling pathway in OSCC and its impact on prognosis. It found that overexpression of phosphorylated mTOR (p-mTOR) is significantly associated with poor survival outcomes. Interestingly, the study did not find a strong correlation between IGFR1 expression and patient prognosis, suggesting that p- mTOR, rather than IGFR1, might be a more reliable predictor of OSCC progression. (27).

Our research emphasizes the importance of IGFR1 as a key marker for OSCC progression. However, this study indicates that the PI3K/AKT/mTOR pathway may play a more central role in influencing OSCC behavior and that p-mTOR expression could be a more effective marker for prognosis than IGFR1. This challenges the notion of using IGFR1 alone as a prognostic marker and suggests that further research should focus on evaluating the role of p-mTOR and its related pathways.

5 | CONCLUSION

From this study, we concluded that:

- IGFR1 is known to be involved in cell proliferation, differentiation, and survival.
- Its overexpression in OSCC making it a potential therapeutic target.
- CD34 can serve as an important prognostic marker in OSCC, with higher expression levels indicating tumors in patients at higher risk.
- The consistent increase in IGFR1 expression across different OSCC grades supports that it may be a potential biomarker for OSCC prognosis as we got observations from Real-time PCR.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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