https://doi.org/ 10.33472/AFJBS.6.5.2024. 5841-5855

African Journal of Biological Sciences

PROSPECTIVE STUDY OF BIOCHEMICAL AND ANTIOXIDANTS STATUS IN ORAL SQUAMOUS CELL CARCINOMA PATIENTS RECEIVING PACLITAXEL

Benish Haider[1], Yrysbaev Erzamat Yrysbaevich[2], *Hafiz Muhammad Arsalan[3], Uraiimov Elaman Toktobolotovich[4], Akmatova Elmira Kazakbaevna[5], Muhammad Shoaib Ramzan[6], Neelam Farheen[7]

1 – School of Biochemistry, Minhaj University Lahore-Pakistan.

2 – Department of Clinical Biochemistry and Pathophysiology, OSH State University, OSH-Kyrgyzstan.

3– International Faculty of General Medicine, Altamimi International Medical University, Bishkek-Kyrgyzstan.

4 – Department of Pathology, International School of Medicine, International University of Kyrgyzstan, Bishkek-Kyrgyzstan.

5 – Department of Fundamental Disciplines, International School of Medicine, International University of Kyrgyzstan, Bishkek-Kyrgyzstan.

6 – Department of Microbiology, Adan Hospital-Kuwait.

7 – Department of Biochemistry, Green International University, Lahore-Pakistan.

Corresponding Author: Dr. Hafiz Muhammad Arsalan (Email: dr.arsalan19@gmail.com)

Article History
Volume 6, Issue 5, 2024
Received: 09 May 2024
Accepted: 17 May 2024
doi: *10.33472/AFJBS.6.5.2024. 5841-5855*

ABSTRACT

Background: Oral squamous carcinoma cell is generally defined as an oral neoplastic disorder in the mouth. Hence, the OSCC originate from the epithelial lining, which is present in the oral cavity. Most cancers begin with lips and mouths in squamous cells, thin and flat cells that affect the lips and the oral cavity. So that's why they are called squamous cell carcinomas.

Objective: To evaluate the correlation between OSCC, paclitaxel and biochemical status.

Methodology: 5.0 ml venous blood sample of 60 patients of OSCC treated with Paclitaxel and 50 Blood sample of Healthy individuals was taken in clotted gel vial from Oral and Maxillofacial department, Mayo hospital Lahore. Blood was further processed for the estimation of Reduce Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Estimation of Nitric oxide (NO), Estimation of micronutrients (Vitamin A, Vitamin C and Vitamin E), Estimation of AOPPs, Estimation of AGEs, Liver Functions tests (LFTs), Lipid Profile, Estimation of urea and creatinine and Electrolytes concentration by flame photometer (Na+ and K+) by kit method.

Results: The serum NO level in diseased person is 0.46 ± 0.62 while in controlled healthy person is 4.21 ± 1.17. The serum NO is significant statistically ($p= 0.001 \leq 0.05$). Level of MDA in diseased person is 0.64 ± 0.45 while in controlled healthy person is 3.18 ± 0.11.

Hence, MDA is significant statistically ($p= 0.000 \leq 0.05$). Level of CAT in diseased person is 11.11 ±0.12 while in controlled healthy person is 1.31 ± 1.08. So CAT is significant statistically ($p= 0.000 \leq 0.05$).GSH level in diseased person is 0.72 ± 0.29 while in controlled healthy person is 4.00 ± 0.41, so GSH is significant statistically ($p= 0.000 \leq 0.05$).SOD level in diseased person is 0.40 ± 0.04 while in controlled healthy person is 3.31 ± 1.08. SOD is significant statistically ($p= 0.000 \leq 0.05$). Vitamin A level in diseased person is 10.25±2.16 while in controlled healthy person is108.08 ± 6.12. Vitamin A is significant statistically ($p= 0.000 \leq 0.05$).Vitamin E level in diseased person is 1.37±0.48 while in controlled healthy person is 9.06 ± 0.35, so vitamin E is significant statistically ($p= 0.000 \leq 0.05$).

Conclusion: The oral squamous cell carcinoma is very pronounced topic. In this cohort study, we found a strong relationship and correlation of lipid peroxidation and biochemical response in patients, receiving paclitaxel. OSCC is very common among all the cancers in the world.

Key Words: OSCC, Paclitaxel, SOD, GSH, MDA, CAT.

INTRODUCTION

Oral squamous carcinoma cell is generally defined as an oral neoplastic disorder in the mouth (1). Hence, the OSCC originate from the epithelial lining, which is present in the oral cavity (2). Most cancers begin with lips and mouths in squamous cells, thin and flat cells that affect the lips and the oral cavity. So that's why they are called squamous cell carcinomas (3). Cancer cells can grow in deeper tissues when cancer develops. In general, SCC develops in areas with leukoplakia (white spots of non-gummy cells) (1).

Among most of the cancers, the OSCC is the sixth most usual cancer in the world (4). Squamous cell carcinoma in the mouth accounts for more than 90% of all oral cancers (5). A "precancerous" condition appears to constitute more than 50% of tumors, which often occurs with abnormal disorders, such as leukoplakia, oral lichen planus and oral submucosal fibrosis (1). The durability of OSCC is 5 years. Its existence has been remained over about 50% since for last 30 years (6). OSCC is somewhat complicated when surrounding factors, infection due to virus and genetic exchanges lead to the cancerous condition (7). The genetic alternation includes, deletion, point mutations, promoter methylation, gene amplification of oncogenes, inactivate tumor suppressor genes, leading to malignant disease (8). Approximately, about all of the mouth cancers, the squamous cell carcinoma (SCC) is 90% (9). The oral SCC happens due to the usage of tobacco, betel quid (BQ) as well as also the continuous intake of alcoholic beverages. Moreover, the high exposure infection of Human Papilloma Virus (HPV) genotypes, and the less

intake of fresh fruits and vegetables have also been incriminated in the oral SCC (4, 5). The tumors were usually diagnosed at the ages between 50 and 79 years, age-related disease SCC is oral cavity, with approximately 90% to 95% of cases existing personally above 40 years (10). There are some techniques which contribute to the diagnosis of OSCC which includes: vital staining, light based detection systems, imaging technique, molecular analyses and cytological technique (11).

OSCC can be viewed anywhere in mouth. The very popular areas are the tongue and the surface of the mouth. The oral cavity comprises the following elements: the first two thirds of the tongue, the ginviva (gums), oral mucous membranes (interlining of the cheeks) and floor (low) of the oral tongue, hard palate (mouth roof), retromolar trigone (the small area behind the teeth of the wisdom) (1). About 30-35% of the tumor (OSCC) present in tongue, 20-25% of the gums, 5 to 7% of the oral floor, 4 to 6% of the soft palate and only 2 to 3% of the cheeks(12).The major causes for oral SCC are chronic humorous factors such as tobacco, betel quid (QB) and daily consumption of alcoholic beverages. Although the main risk factors are smoking and alcohol consumption. OSCC works with the Indian subdivision with a very high frequency and prevalence, probably because of the usage of chomping tobacco, betel quid and seed of betel palm on daily base (13).

Oxidation stress is a cellular condition, described by the production of reactive oxygen species (ROS) that have reduced the protection of antioxidants. Superoxide (O_2) radicals, nitrous oxide (NO), hydroxyl radicals (OH) and hydrogen peroxide (H2O2), which are the Reactive Oxygen species (ROS) play a key role as in the development of cancer in humans. ROS can cause many damages in DNA structure such as alternation in bases, breakage of strands, damage to tumor suppressor genes and improved appearance of proto-oncogenes (14). A mutation that induces ROS can lead to protein damage and an attack on lipids, which then triggers lipid peroxidation. ROS mainly attack the polyunsaturated fatty acids (PUFA), which is mainly present in the membrane lipids. In addition, the breakdown of these per oxidized lipids creates a variety of end products, including lipid hydroperoxides (LHP) and malondialdehyde (MDA). Levels of these peroxide lipids generally indicate the degree of lipid peroxidation and act as markers of cell damage due to free radicals (15).

MATERIAL AND METHODS

Place of Work

The whole experimental work was done in the Biochemistry Lab, School of Biochemistry and medical Lab Technology, Faculty of Allied Health Sciences, Minhaj University Lahore.

Blood/Data Collection

Blood samples (5.0 ml) of 60 Diagnosed Oral Squamous Carcinoma Cell patients (OSCC) and blood samples (5.0 ml) of 50 healthy individuals as a control group was taken from vein in clotted gel vial from Oral and Maxillofacial department, Mayo Hospital Lahore. Blood was further processed for the estimation of Lipid Profile, Liver Function Tests (LFT's) by kit methods, Reduced Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Estimation of Nitric oxide (NO), Estimation of micronutrients (Vitamin A, Vitamin C and Vitamin E) and Electrolytes concentration by flame photometer (Na^+, K^+, Ca^{++}, Mg^{++}).

Serum Separation

Blood samples were centrifuged at 4000 rpm for 10 minutes and serum was separated. The serum was preserved at -20°C till further analysis.

Estimation of Superoxide Dismutase (SOD)

SOD was measured and estimated by spectrophotometric method (16).

Determination of Thiobarbituric Acid Reactive Substances (TBARS) in Tissues

Malondialdehyde (MDA) was measured by spectrophotometric method (17).

Estimation of Catalase (CAT)

CAT was measured by spectrophotometric method (18).

Determination of Glutathione (GSH)

GSH was determine by the process of Moron (19).

Determination of Nitric Oxide (NO)

Nitrite concentration is typically measured by a well-known method such as colorimetric Griess assay (20).

Estimation of Vitamin C (VIT C)

Ascorbic acid (VIT C) was analyzed by the method described by Roe and Keuther (21).

Estimation of Vitamin A (VIT A)

Vitamin A (Tocopherol) was estimated by the Emmutir-Engel reaction as reported by Rosenberg (22).

RESULTS

Table 1	**Comparison of Anti-oxidant Biomarkers between control and Oral Squamous Cell Carcinomas patients receiving Paclitaxel**

PARAMETERS	**CONTROL (n= 50) Mean± S.D**	**DISEASED (n=60) Mean± S.D**	**p –value (P≤0.05)**
NO	4.21 ± 1.17	0.46 ±0.62	0.001
MDA (nmol/ml)	3.18 ± 0.11	0.64±0.45	0.000
CAT (µg/dL)	1.31 ± 1.08	11.11 ±0.12	0.000
GSH (µg/dL)	4.00 ± 0.41	0.72 ± 0.29	0.000
SOD (µg/dL)	3.31 ± 1.08	0.40±0.04	0.000
AGE'S (kU/I)	1.07 ± 0.35	1.36 ± 0.44	0.000
AOPP (µmol/dL)	6.00 ± 2.00	0.41±0.19	0.000

NORMAL RANGE; MDA = 1-3 nmol/ml, SOD = 0.1-0.6 nmol/ml, GSH =8-12mg/dl, and Catalase =1-5µmol/mol of protein
Significance level ≤ 0.05

Data presented in **table 1** explain the clear picture of different biomarkers of anti-oxidant status check estimated in OSCC receiving paclitaxel. From the table it is revealed that serum NO level in diseased person is **0.46 ± 0.62** while in controlled healthy person is **4.21 ± 1.17**, which explained that serum NO level in OSCC patients reduced. Data also predicted that serum NO is significant statistically **(p= 0.001 ≤ 0.05).**From the table it is predicted that MDA level in diseased person is **0.64 ± 0.45** while in controlled healthy person is **3.18 ± 0.11,** which explained that MDA level in OSCC patients decreased. Data also predicted that MDA is significant statistically **(p= 0.000 ≤ 0.05).** From the table it is revealed that CAT level in diseased person is

11.11 ±0.12 while in controlled healthy person is **1.31 ± 1.08**, which explained that CAT level in OSCC patients increased. Data also predicted that CAT is significant statistically **(p= 0.000 ≤ 0.05).**From the table it is predicted that GSH level in diseased person is **0.72 ± 0.29** while in controlled healthy person is **4.00 ± 0.41**, which explained that GSH level in OSCC patients decreased. Data also predicted that GSH is significant statistically **(p= 0.000 ≤ 0.05).**From the table it is revealed that SOD level in diseased person is **0.40 ± 0.04**while in controlled healthy person is **3.31 ± 1.08**, which explained that SOD level in OSCC patients declined. Data also predicted that SOD is significant statistically **(p= 0.000 ≤ 0.05).**From the table it is predicted that AGE'S level in diseased person is **1.36 ± 0.44** while in controlled healthy person is **1.07 ± 0.35**, which explained that AGE'S level in OSCC patients increased. Data also predicted that AGE'S is significant statistically **(p= 0.000 ≤ 0.05).** From the table it is estimated that AOPP level in diseased person is **0.41 ± 0.19** while in controlled healthy person is **6.00 ± 2.00**, which explained that AOPP level in OSCC patients declined. Data also predicted that AOPP is significant statistically **(p= 0.000 ≤ 0.05).**

Table 2	**Comparison of micro-nutrients in between control and Oral Squamous Cell Carcinomas patients receiving Paclitaxel**

PARAMETERS	**CONTROL (n=50) Mean± S.D**	**DISEASED (n=60) Mean± S.D**	**p-VALUE (P≤ 0.05)**
Vitamin A (µg/ml)	108.08 ± 6.12	10.25±2.16	0.000
Vitamin C (µg/ml)	5.06 ± 1.00	2.43±1.12	0.000
Vitamin E (µg/ml)	9.06 ± 0.35	1.37±0.48	0.000

Significant level ≤ 0.05

Data presented in table 2 explain the clear picture of different micro-nutrients status check estimated in OSCC receiving paclitaxel. From the table it is revealed that vitamin-A level in diseased person is **10.25±2.16** while in controlled healthy person is **108.08 ± 6.12**, which explained that vitamin A level in OSCC patients reduced. Data also predicted that vitamin A is

significant statistically **(p= 0.000 ≤ 0.05).** From the table it is predicted that vitamin C level in diseased person is **2.43±1.12** while in controlled healthy person is **5.06 ± 1.00,** which explained that vitamin C level in OSCC patients decreased. Data also predicted that vitamin C is significant statistically **(p= 0.000 ≤ 0.05).** From the table it is estimated that vitamin E level in diseased person is **1.37±0.48** while in controlled healthy person is **9.06 ± 0.35**, which explained that vitamin E level in OSCC patients declined. Data also predicted that vitamin E is significant statistically **(p= 0.000 ≤ 0.05).**

Table 3	**Comparison of LFT's parameter in between control and Oral Squamous Cell Carcinomas patients receiving Paclitaxel**

Parameters	**Control (n=50) Mean±S.D**	**Diseased (n=60) Mean±S.D**	**P-Value (p ≤ 0.05)**
Bilirubin (ml/dL)	0.40 ± 0.31	0.46±0.11	0.000
ALT (U/L)	29.03±5.31	44.36±7.87	0.000
AST (U/L)	28.11±8.23	42.68±8.97	0.000
ALP (IU/L)	139±16.10	186.60±22.77	0.000
Normal Ranges:bilirubin= 0.2- 0.8ml/dL, AST=<35U/L, ALT=<35 U/L, ALP= 44-147			

Data presented in table 3 explain the clear picture of different LFT's parameter status check estimated in OSCC receiving paclitaxel. From the table it is revealed that bilirubin level in diseased person is**0.46±0.11** while in controlled healthy person is**0.40 ± 0.31**, which explained that bilirubin level in OSCC patients increased. Data also predicted that bilirubin is significant statistically **(p= 0.000 ≤ 0.05).** From the table it is predicted that ALT level in diseased person is **44.36±7.87**while in controlled healthy person is **29.03±5.31,** which explained that ALT level in OSCC patients increased. Data also predicted that ALT is significant statistically **(p= 0.000 ≤ 0.05).** From the table it is estimated that AST level in diseased person is **42.68±8.97** while in controlled healthy person is **28.11±8.23**, which explained that AST level in OSCC patients

increased. Data also predicted that AST is significant statistically **(p= 0.000 ≤ 0.05).**From the table it is estimated that ALP level in diseased person is**186.60±22.77** while in controlled healthy person is **139±16.10**, which explained that ALP level in OSCC patients increased. Data also predicted that ALP is significant statistically **(p= 0.000 ≤ 0.05).**

Table 4	**Comparison of Lipid profile in between control and Oral Squamous Cell Carcinomas patients receiving Paclitaxel**

Parameters	**Control (n=50) Mean±S.D**	**Diseased (n=60) Mean±S.D**	**P-Value (p ≤ 0.05)**
Tg (mg/dl)	168.12±19.23	154.16±28.89	0.000
Cholesterol (mg/dl)	187.45±23.34	184.24±19.72	0.000
Reference Range: Tg= <150mg/dl, Cholesterol= <200mg/dl.			

Data presented in table 4 explain the clear picture of different Lipid profile parameter status check estimated in OSCC receiving paclitaxel. From the table it is revealed that Tg level in diseased person is **154.16±28.89** while in controlled healthy person is**168.12±19.23**, which explained that Tg level in OSCC patients decreased. Data also predicted that bilirubin is significant statistically **(p= 0.000 ≤ 0.05).** From the table it is predicted that cholesterol level in diseased person is **184.24±19.72** while in controlled healthy person is **187.45±23.34,** which explained that cholesterol level in OSCC patients increased. Data also predicted that cholesterol is significant statistically **(p= 0.000 ≤ 0.05).**

Table 5	**Comparison of RFT's parameter in between control and Oral Squamous Cell Carcinomas patients receiving Paclitaxel**

Parameters	**Control (n=50) Mean±S.D**	**Diseased (n=60) Mean±S.D**	**P-Value (p ≤ 0.05)**

Urea (mg/dL)	43.12 ± 2.12	39.32±6.90	0.000
Creatinine (mg/dL)	1.13 ± 0.12	0.84±0.14	0.000
Normal Ranges: Urea = 7-20mg/dL ,Creatinine = 0.6-1.2 mg/dL.			

Data presented in table 5 explain the clear picture of different RFT's parameter status check estimated in OSCC receiving paclitaxel. From the table it is revealed that urea level in diseased person is**39.32±6.90** while in controlled healthy person is**43.12 ± 2.12**, which explained that urea level in OSCC patients decreased. Data also predicted that urea is significant statistically **(p= 0.000 ≤ 0.05).** From the table it is predicted that creatinine level in diseased person is **0.84±0.14** while in controlled healthy person is **1.13 ± 0.12,** which explained that creatinine level in OSCC patients decreased. Data also predicted that creatinine is significant statistically **(p= 0.000 ≤ 0.05).**

Table 6	**Pearson Correlation among different parameters**

Parameters	**Correlation**	**P value**
AGE'S vs AOPP	-0.467*	0.021
SOD vs Vitamin C	0.665**	0.000
NO vs Triglyceride	0.539**	0.007
AGE'S vs AST	0.581**	0.003
AGE'S vs Urea	0.458*	0.024
AOPP vs AST	-0.474*	0.019
AOPP vs Creatinine	-0.429*	0.037
Vitamin A vs AST	-0.483*	0.017
Vitamin C vs Urea	0.405*	0.050
Vitamin C vs Creatinine	0.461*	0.023

Bilirubin vs Urea	0.528**	0.007
Bilirubin vs Cholesterol	0.485*	0.014
ACT vs AST	0.397*	0.050
Urea vs Creatinine	0.606**	0.001
Urea vs Cholesterol	0.481*	0.015
Creatinine vs Cholesterol	0.554**	0.004

DISCUSSION

It was recommended that periodic damage to cellular DNA due to reactive oxygen species which is produced by tobacco consumption and accumulation has been suggested to contribute in oral cancer. Disturbances in the balance between ROS production and protective antioxidant efficiency have led to oxidative stress. Therefore, in oxidative stress there is excessive production of ROS or there is a significant reduction or lack of antioxidant defense. It has been reported that there is a low concentration of ROS to stimulate cell proliferation, where high levels can damage proteins, nucleic acid, cell membrane and stimulate cytoxicity and cell death. By-products of lipid peroxidation cause significant changes in the structural integrity and function of cell membranes.

The decomposition of this peroxide lipid leads to a production of variety of end products such as MDA. Therefore, the level of MDA indicates the degree of peroxide and acts as a sign of cellular damage. The increase in MDA may be due to excessive free radical formation due to degradation of polyunsaturated fatty acids contained in membranes or may occur due to insufficient release of the poor cellular antioxidant system.

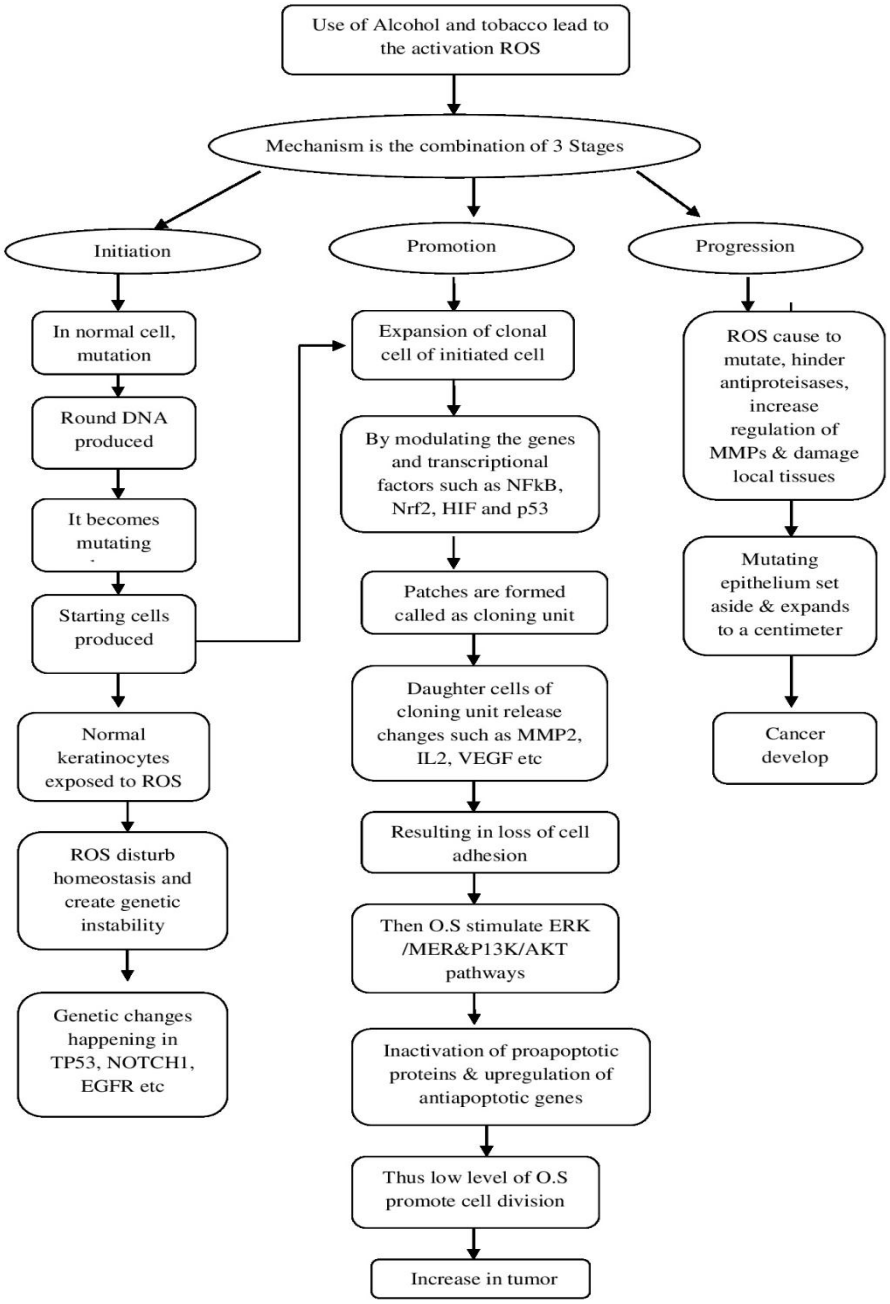

Figure 2: Flowchart Mechanism of action of Oxidative stress/ROS induced Oral Squamous Cell Carcinoma (OSCC)

Data present in table no 6 explain the correlation exist between different parameters estimated in OSCC patients receiving paclitaxel.

Table shows that inverse correlation between **AGES'S and AOPP(r=-0.467*)** and its p value is **(p=0.021)**. It is demonstrated that by increase in the level of AGE'S, the level of AOPP will decreased and vice versa.

Table shows that direct correlation between **SOD and Vitamin C (r=0.665**)** and its p value is **(p=0.000)**. It is demonstrated that by increase in the level of SOD, the level of vitamin C will be also increased and vice versa.

Table shows that direct correlation between **NO and Triglyceride (r=0.539**)** and its p value is **(p=0.007)**. It is demonstrated that by increase in the level of NO, the level of triglyceride will be also increased and vice versa.

Table shows that direct correlation between **AGE'S and AST (r=0.581**)** and its p value is **(p=0.003)**. It is demonstrated that by increase in the level of Age's, the level of AST will be also increased and vice versa.

Table shows that direct correlation between **AGE'S and Urea (r= 0.458*)** and its p value is **(p=0.024)**. It is demonstrated that by increase in the level of AGE'S, the level of Urea will be also increased and vice versa.

Table shows that inverse correlation between **AOPP and AST (r=-0.474*)** and its p value is **(p=0.019)**. It is demonstrated that by increase in the level of AOPP, the level of AST will decreased and vice versa.

Table shows that inverse correlation between **AOPP vs Creatinine (r=-0.429*)** and its p value is **(p=0.037)**. It is demonstrated that by increase in the level of AOPP, the level of creatinine will decreased and vice versa.

Table shows that inverse correlation between **Vitamin A and AST (r= -0.483*)** and its p value is **(p=0.017)**. It is demonstrated that by increase in the level of vitamin A, the level of AST will decreased and vice versa.

Table shows that direct correlation between **Vitamin C and Urea (r= 0.405*)** and its p value is **(p=0.050)**. It is demonstrated that by increase in the level of vitamin C, the level of Urea will be also increased and vice versa.

Table shows that direct correlation between **Vitamin C and Creatinine (r=0.461*)** and its p value is **(p=0.023)**. It is demonstrated that by increase in the level of vitamin C, the level of creatinine will be also increased and vice versa.

Table shows that direct correlation between **Bilirubin and Urea (r=0.528**)** and its p value is **(p=0.007)**. It is demonstrated that by increase in the level of bilirubin, the level of Urea will be also increased and vice versa.

Table shows that direct correlation between **Bilirubin and Cholesterol (r= 0.485*)** and its p value is **(p=0.014)**. It is demonstrated that by increase in the level of bilirubin, the level of cholesterol will be also increased and vice versa.

Table shows that direct correlation between **ACT and AST (r= 0.397*)** and its p value is **(p=0.050)**. It is demonstrated that by increase in the level of ACT, the level of AST will be also increased and vice versa.

Table shows that direct correlation between **Urea and Creatinine (r=0.606**)** and its p value is **(p=0.001)**. It is demonstrated that by increase in the level of urea, the level of creatinine will be also increased and vice versa.

Table shows that direct correlation between **Urea and Cholesterol (r= 0.481*)** and its p value is **(p=0.015)**. It is demonstrated that by increase in the level of urea, the level of cholesterol will be also increased and vice versa.

Table shows that direct correlation between **Creatinine and Cholesterol (r= 0.554**)** and its p value is **(p=0.004)**. It is demonstrated that by increase in the level of creatinine, the level of cholesterol will be also increased and vice versa.

CONCLUSION

The oral squamous cell carcinoma is very pronounced topic. In this cohort study, we found a strong relationship and correlation of lipid peroxidation and biochemical response in OSCC patients, receiving paclitaxel. OSCC is very common among all the cancers in the world. Present study concluded that anti-oxidant activity, micro nutrients decreased in oral cancer patients. LFT and RFT increased due to the cytotoxic drug (paclitaxel) which leads to the progression of OSCC.

REFERENCES

1. Schepman, K., Meij, E., Smeele, L., and Waal, I. (1999). Concomitant leukoplakia in patients with oral squamous cell carcinoma. *Oral Diseases*, 5(1), 206–209.
2. Cheng, Y and Wright, J. (2011). Advances in diagnostic adjuncts for oral squamous cell carcinoma. *The Open Pathology Journal*, 5(1), 3–7.
3. Lingen, M. W, Kalmar, J. R, Karrison, T, and Speight, P. M (2008). Critical evaluation of diagnostic aids for the detection of oral cancer *Oral Oncology, 44(1),* 10–22.
4. Shah, J P, and Gil, Z (2009). Current concepts in management of oral cancer Surgery *Oral Oncology,* 45(4-5), 394–401.

5. Chen, Y. K, Huang, H. C, Lin, L. M, and Lin, C. C (1999). Primary oral squamous cell carcinoma: an analysis of 703 cases in southern Taiwan *Oral Oncology*, 35(2),173–179.
6. Bhatia, N., Lalla, Y., Vu, A. N, and Farah, C. S (2013). Advances in Optical Adjunctive Aids for Visualisation and Detection of Oral Malignant and Potentially Malignant Lesions *International Journal of Dentistry*, 2013(1), 1–17.
7. Ellerman, C, and Bang, O (1908). Experimentelle Leuka¨mie bei Hu¨hnern Cent Bakteriol 46(1), 595-609
8. Leemans, C. R, Braakhuis, B.J, and Brakenhoff, R.H (2011). The molecular biology of head and neck cancer *Nature Review Cancer*, 11(1), 9–22.
9. Attar, E, Dey, S, Hablas, A, Seifeldin, I A, Ramadan, M, Rozek, L S, and Soliman, A S. (2010). Head and neck cancer in a developing country: A population-based perspective across 8years *Oral Oncology*, 46(8), 591–596.
10. Howell, R E, Wright, B A, and Dewar, R. (2003). Trends in the incidence of oral cancer in Nova Scotia from 1983 to 1997 *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 95(2), 205–212.
11. Carreras-Torras, C, and Gay-Escoda, C. (2015). Techniques for early diagnosis of oral squamous cell carcinoma: Systematic review *Oral Medicine and Pathology Publication Types: Review*, *20*(3), 305-315.
12. Laramore, G. E, Scott, C. B, Al-Sarraf, M, Haselow, R. E, Ervin, T. J, Wheeler, R, and Campbell, B. H. (1992). Adjuvant chemotherapy for resectable squamous cell carcinomas of the head and neck: report of intergroup study 0034 *International Journal of Radiation Oncology Biology Physics*, *23*(4), 705-713.
13. Attar, E., Dey, S., Hablas, A., Seifeldin, I. A., Ramadan, M., Rozek, L. S, and Soliman, A. S. (2010). Head and neck cancer in a developing country: a population-based perspective across 8 years *Oral oncology*, *46*(8), 591-596.
14. Cerutti, P. A. (1994). Oxy-radicals and cancer *The Lancet*, 344(8926), 862-863.
15. Moncada, S R M J. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology *Pharmacol rev*, *43*, 109-142
16. Warnakulasuriya, K A A S, Johnson, N W, Linklater, K M, and Bell, J (1999) Cancer of mouth, pharynx and nasopharynx in Asian and Chinese immigrants resident in Thames regions *Oral Oncology*, *35*(5), 471-475

17. Warnakulasuriya, K A A S, and Ralhan, R. (2007). Clinical, pathological, cellular and molecular lesions caused by oral smokeless tobacco–a review *Journal of oral pathology & medicine*, *36*(2), 63-77.
18. Anonymous. (2003). IARC Global cancer rates could increase by 50% to 15 million by 2020 IARC Press, Release No 145.
19. Solt, D. B., Chang, K. W., Helenowski, I, and Rademaker, A. W. (2003). Phenethyl isothiocyanate inhibits nitrosamine carcinogenesis in a model for study of oral cancer chemoprevention *Cancer letters*, *202*(2), 147-152.
20. Axéll, T. (1987). Occurrence of leukoplakia and some other oral white lesions among 20 333 adult Swedish people *Community dentistry and oral epidemiology*, *15*(1), 46-51.
21. Petti, S, Masood, M, Messano, G. A, and Scully, C. (2013). Alcohol is not a risk factor for oral cancer in nonsmoking, betel quid non-chewing individuals A meta-analysis update*Annali DiI giene*,*25*(1), 3-14.
22. Anonymous. (1985). International Agency for Research on Cancer IARC monographs on the evaluation of carcinogenic risks to humans Tobacco habits other than smoking; betel-quid and areca- nut chewing; and some areca- nut- derived nitrosamines Vol 37 Lyon: IARC. p 188.