RESEARCH ARTICLE

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Evaluation and Correlation of 17β-Estradiol in Blood and Estrogen Receptor α (ERα) and Estrogen Receptor β(ER β) in Tissue of Oral Squamous Cell Carcinoma

Eesha Thakare[1]*, Minal Chaudhary[2], Madhuri Gawande[3], Amol Ramchandra Gadbail[4], Prajakta Zade[5]

Abstract

Objective: Evaluation and correlation of 17β-Estradiol(E2) in blood and Estrogen Receptor α (ERα) and Estrogen Receptor β(ER β) in tissue of four groups divided as normal control subjects (group I), active control subjects (group II), premalignant(leukoplakia) (group III) and OSCC patients (Group IV). **Methods:** Subjects were divided in four groups mentioned previously and evaluated individually for 17β-estradiol(E2) in blood and ERα & ER β in tissue. The blood samples were evaluated by chemiluminescence assay and tissues samples were evaluated by Real-Time PCR for tissue analysis. Analysis of variance (ANOVA) was applied for quantitative evaluation of variables in each group followed by TukeyB test. Pearson Correlation Coefficient was used to study the correlation between the variables. Survival analysis was calculated by Kaplan-Meier Survival Analysis. **Results:** The results are indicative of statistically significant increased levels of E2 in the 4 groups by analysis of variance (p value-0.0327) followed by TukeyB test with statistically significant difference between Group VI and I (sig value-0.008) and Group VI and II (sig value-0.029). The Pearson correlation coefficient demonstrated that the mean levels of Estradiol are positively correlated with mean levels of ER α (p value- 0.030). Results did not indicate an association between survival and expression of E2, ER α and ER β. **Conclusion:** The levels of E2 can be used as a marker in predicting the progression of disease from normal tissue not exposed to tobacco to normal tissue exposed to tobacco to premalignant to OSCC. The ERα in tissue is positively correlated with the increased levels of E2 in serum, so ERα expression in tissue along with E2 in serum could be used to identify the subsets of patients with higher risk of developing OSCC, especially those subjects with established tobacco habit but no appreciable change in oral mucosa.

Keywords: Estradiol- estrogen receptors- OSCC- Leukoplakia- tobacco

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Introduction

Head & neck squamous cell carcinoma (HNSCC) in 2018 accounted for 450,000 deaths and around 890,000 new cases with an anticipated rise of 30%, annually accounting around 1.08 million cases as new by 2030 [1].

The myriads of etiologies causing OSCC enlists numerous agents like tobacco, alcohol, betel quid and arecanut with history of long-term usage [2]. But when associated with smokeless tobacco, its usually Oral squamous cell carcinoma (OSCC) [3]. Tobacco (smokeless or smoking) is the prime reason in the etiology of OSCC [4]. Tobacco habit varies in consumption from either in smoking or smokeless form (with slaked lime or with areca nut products) [1], while in Vidarbha region of Maharashtra (India) it is consumed mostly in the form of gutka, kharra, nus etc which is distinct and different from other forms of chewable tobacco consumed in other regions.

Oral squamous cell carcinoma has become the most common carcinoma amongst Indian males and India accounts for around 60% new cases globally [5]. Although having numerous advances in diagnosis and treatment along with awareness of population towards OSCC, a survival rate not more than 65% has been reported [4]. Though the prime etiological factor for OSCC is still tobacco, now it is recognized as a potential risk in carcinoma of breast too because the mode of activation of Estrogen receptors by tobacco (nicotine) is found to be

*[1]Department of Oral Pathology and Microbiology, Nanded Rural Dental College, Nanded, Maharashtra, India. [2]Datta Meghe Institute of Medical Sciences (Deemed to be university), Nagpur (MS), India. [3]Department of Oral Pathology & Microbiology, Datta Meghe Institute of Medical Sciences (Deemed to be university), Nagpur (MS), India. [4]Department of Dentistry, Shree Bhausaheb Hire Government Medical College and Hospital, Dhule, Maharashtra, India. [5]Department of Dentistry, Indira Gandhi Medical college and Hospital, Nagpur, Maharashtra, India. *For Correspondence: eeshathakare254@gmail.com*

similar to that of estradiol [6]. The effect of estrogen is similar in oral mucosa as that of vaginal mucosa due to presence of ERs [7].

Estrogen exhibits its effect in the form of hormones like Estradiol (E2), estriol, estrone and estretone [8], amongst them E2 is produced the highest and its effects are mediated by estrogen receptors (ERs) basically ER α & ER β. Estrogen not only plays a vital role in reproductive organs and abilities in females and males, but also numerous carcinomas of the body ranging from breast, uterus, prostate, lung, gastric [9].

Many researchers like Egloff et al and Collela et al reported that ER α was expressed more in HNSCC tumor tissue than normal adjacent tissue [10, 11]. Grim et al studied the ER α expression in oral precursor region along with HNSCC tissue with similar expression of ER α in both the tissues [12]. Remenar et al evaluated E2 in OSCC reporting raised levels of E2 in OSCC patients compared to others [13]. While Ishida et al evaluated ER α and ER β in OSCC reporting predominant expression of ER β [14], while Shatalova et al reported E2 inhibits apoptosis and helps in proliferation of OSCC [15]. This evidence suggests that estrogen may play a role in oral carcinogenesis, but review in literature suggests that a knowledge gap does exist as E2 levels along with correlation to ERs(especially ER α and ER β) in leukoplakia and OSCC has not been reported. So this study was designed with the aim of quantifying estrogen in blood (in the form of E2) and in tissue (in the form of ER α and ER β) and finding a possible correlation of levels of E2 and risk of oral carcinogenesis. This is the first study to report the correlation of E2 in serum to ERs in tissues of tobacco associated and not associated oral tissues along with OSCC.

Aim and Objective

Evaluation, comparison and correlation of 17β-Estradiol in blood and Estrogen Receptor α (ERα) and Estrogen Receptor β(ER β) in tissue of normal control subjects(normal without the habit of tobacco- NWOH), active control subjects (normal with habit of tobacco-NWH), premalignant(leukoplakia-P) and Oral squamous cell carcinoma (O) patients.

Study Area and Design

After approval from the Institutional ethical committee, this study was carried out from 2018 to 2021 in the School of Oral Oncology, SPDC, D.M.I.M.S.U, Sawangi, Wardha, along with assistance from PAR laboratory, Trichy. The patients were followed up for survival till April 2023. Subjects were divided in four groups like group I, II, III and group IV and evaluated individually for 17β-estradiol(E2) in blood and ERα & ER β in tissue (Figure 1). As OSCC is a progressive stage from premalignancy to malignancy, this study was designed to study the complete spectrum of normal tissue not exposed to tobacco to tissue exposed to tobacco to premalignant to OSCC. According to this, the groups were designed and considered following the inclusion and exclusion criteria.

Groups along with the Inclusion and Exclusion Criteria

The study was organized in 4 groups as follows (Table 1) and the data presentation flowchart is described in Figure 1.

Materials and Methods

Protocol and procedure for chemiluminescence assay

The blood samples were collected from subjects and centrifuged immediately to obtain the serum samples which were then analyzed by the "electrochemiluminescence (ECL) immunoassay" in the "Cobas e411 analyzer". For result calibration, the Standard reference range of the test method (estradiol: 18.4-11010pmol/L or 5-3000pg/ml) was considered. As per the "EP5-A2 protocol" of the "Clinical and Laboratory Standards institute (CLSI)",

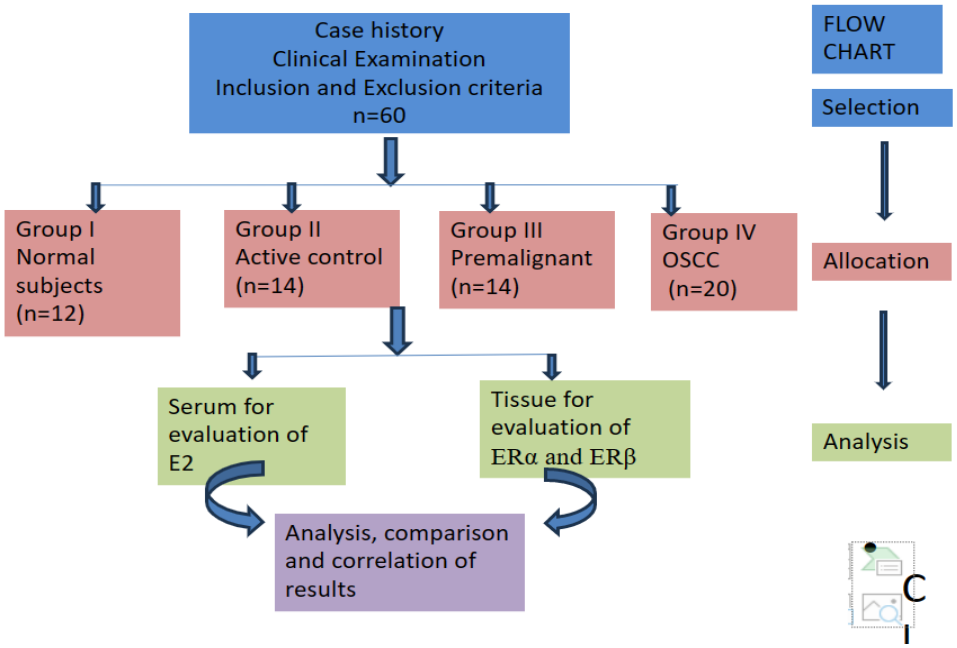

Figure 1. Collaging and Collation of Data

Table 1. Inclusion and Exclusion Criteria for Included Subjects

	Inclusion Criteria	Exclusion Criteria
Group I (Normal Control)	Normal subjects without established disease No habit of Tobacco, areca	Diagnosed with hypertension and diabetes mellitus. Any history of diagnosis of previous malignancy Currently under medication known to influence 'sex hormone level' Habit of Alcohol
Group II (Active control)	No changes seen in mucosa related to tobacco. With habit of Tobacco, areca	
Group III (Premalignant-leukoplakia)	Patients diagnosed as leukoplakia. With habit of Tobacco and areca	
Group IV (OSCC)	Histopathologically diagnosed "oral squamous cell carcinoma". No history of previously treatment with chemotherapy and/or irradiation and surgery. With habit of Tobacco, areca	

the precision in analysis was determined using Elecsys reagents, samples and controls for analysis.

Protocol for Real-Time PCR
RNA Isolation

The fresh tissue (approx.300–500 mg) was immediately immersed in the 2 mL double autoclaved microfuge tube containing 1.3 mL "RNA later" liquid stored for day at –20^{0}C and later at –80^{0}C till time of processing. The RNA was isolated according to 'TRIZOL' (Invitrogen USA) method, as per manufacturers' instructions. The quality and quantity of isolated RNA was calculated by the Labman UV Vis Spectrometer which was determined in agarose gel (1%) with ethidium bromide.

If DNA contamination was detected during "RNA preparation", it was removed thus by the "DNase treatment". For "1U of DNase", the reaction volume of 20 µl was set up. Reverse transcriptase (MMLV) was used to convert a reaction mixture of 1.55 µgm of RNA to cDNA. Reaction mixture (1.55 µgm) of the total RNA was converted to cDNA by using '(MMLV) Reverse transcriptase 1st strand cDNA synthesis kit (Sigma, USA)', as per the manufacturer's protocol. The reverse transcriptase was inactivated, and cDNA & RNA hybrid was denatured. This cDNA was then used as a template to detect ERα and ERβ. Quantitative analysis for gene expression was carried out by real time PCR using SYBR Green chemistry master-mix (Takara Bio, Japan) on a ABI Stepone Plus machine (Thermo Scientific, USA).

The composition of reaction mix for every sample was as follows (Table 2); Each gene, for every sample was assayed in triplicate. The complete processing was carried out under standard protocol.

Table 2. Component Volume of Reaction Mix

Volume	Content
5 µl	2X SYBR Green master-mix
1 µl	cDNA template
0.5 µl	Forward primers (5 µM)
	ESR1F-- CAGATAATCGACGCCAGGGT
	ESR1R-- CATCATTCCCACTTCGTAGCA
0.5 µl	Reverse primers (5 µM)
	ESR2F-- TTGACATGCTCCTGGCAACT
	ESR2R-- TCCATGCCCTTGTTACTCGC
4 µl	Nuclease-free water

Data analysis

The normalized ct value score was calculated after subtracting the ct values of internal control gene (GAPDH) or reference gene from the target gene. The Mean values of these normalized ct values were plotted. The 'fold change' and 'fold variation' in expression of genes was calculated by the 'ΔΔCt' method (inbuilt software).

Results

Sociodemographic data of all patients including history of disease, address, phone number past medical history, clinical presentations, histopathological features and operative/surgical details were recorded. Statistical package "Statistical Package for the Social Sciences (SPSS 21.0)" was used to evaluate the variables. Continuous data was presented by mean and standard deviation. Analysis of variance (ANOVA) was applied for quantitative evaluation of variables in each group and TukeyB test was calculated to find the exact groups showing statistically significant results by Analysis of variance (ANOVA). The observations were correlated by Pearson Correlation Coefficient to study the correlation between the variables. Survival analysis was calculated by Kaplan-Meier Survival Analysis.

The mean ages of subjects included in this study were in range of 41-50 years with 60-70% males in each group (Figure 2). Subject wise quantitative expression of variables is mentioned in following Tables like 3.1, 3.2, 3.3 and 3.4. Analysis of variance demonstrated a statistically significant rise in the mean levels of E2 amongst the groups starting from Group I to II to III to IV, Group IV being the highest (Table 4). Such results were also demonstrated in our preliminary study evaluating the E2 levels only. The results of ANOVA were validated by Tukey B test which indicated that the difference between the levels of E2 in between group IV(OSCC) & I (Normal control) and Group IV(OSCC) & II (Active control) are statistically significant. The alteration in ERα and ERβ mean levels were statistically non-significant. Pearson correlation value (r=0.279) demonstrated

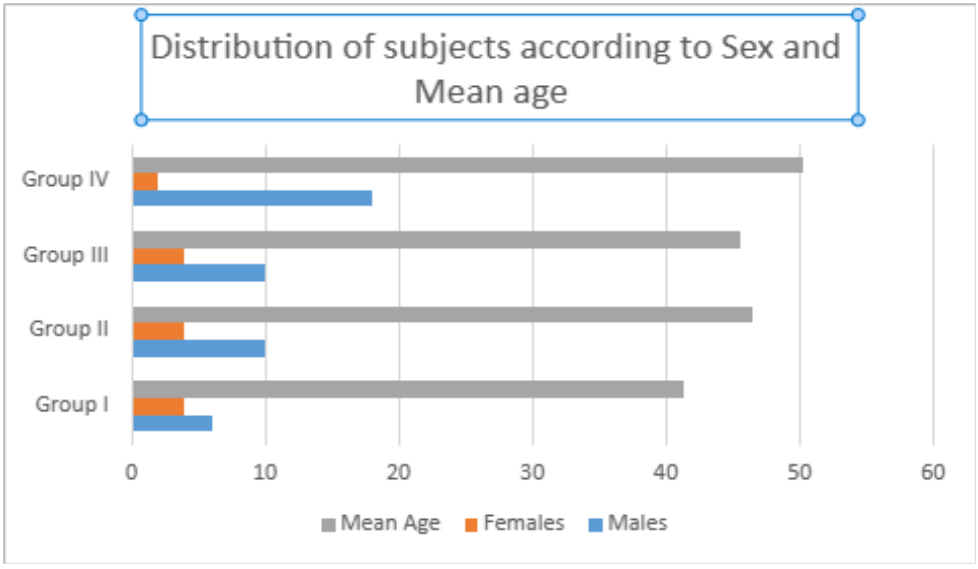

Figure 2. Distribution of Subjects According to Sex and Mean Age

Table 3.1. Quantitative Data of Subjects in Normal Control Group with no Habit of Tobacco and Areca

PATIENT NO.	AGE	ER α	ER β	E2
NWOH-1	32/F	14.9375	ND	30.50
NWOH-2	29/F	32.3809	17.9436	33.9
NWOH-3	52/F	13.268	ND	5
NWOH-4	59/F	28.4128	ND	11
NWOH-5	39/M	18.1784	22.933	23.15
NWOH-6	29/M	ND	23.0516	26.27
NWOH-7	42/M	12.4733	12.1389	32
NWOH-9	48/M	14.9319	18.3616	30.12
NWOH-10	44/M	ND	ND	10
NWOH-11	39/M	17.8384	17.8184	23.2

Note - The details of patients regarding age, mean ct values of ER α and ER β along with serum E2 values included in group I. ND, not detected

positive correlation of ERα with E2 indicating change in ERα per unit changes the E2 value at positive direction with significant P-value<0.01(Figure 3). The correlation between E2 and ERβ could not be established. The results

Table 3.2. Quantitative Data of Subjects in Active Control Group with Habit of Tobacco and Areca

PATIENT NO.	AGE	ER α	ER β	E2
NWH-1	54/M	33.7182	ND	39.6
NWH-2	52/M	20.702	ND	27
NWH-3	38/M	14.2324	ND	48.4
NWH-4	44/M	23.2191	25.1857	28
NWH-5	48/F	9.5972	ND	12
NWH-6	34/F	18.1784	ND	39
NWH-7	56/F	15.5914	ND	-
NWH-8	64/M	ND	ND	40.3
NWH-9	68/F	13.1467	ND	13
NWH-10	33/M	23.2335	21.7624	21.23
NWH-11	49/M	4.7323	15.9093	25.28
NWH-12	56/M	ND	2.1804	21.9
NWH-13	32/M	24.4047	25.3386	33.32
NWH-14	24/M	ND	10.4432	39.77

Note - The details of patients regarding age, mean ct values of ER α and ER β along with serum E2 values included in group II. ND, not detected

Table 3.3 Quantitative Data of Patients in Premalignant Group (Leukoplakia)

PATIENT NO.	AGE	ER α	ER β	E2
P-1	41/M	ND	16.0476	19.5
P-2	36/F	23.0366	27.2675	136.4
P-3	38/M	9.979	ND	16.3
P-4	53/M	31.078	ND	43.22
P-5	38/M	10.5651	ND	16.23
P-6	40/M	6.5755	ND	48.8
P-7	37/F	24.4047	ND	32
P-8	45/M	6.3905	ND	40.15
P-9	68/F	11.1804	ND	-
P-10	42/M	5.0484	ND	25.8
P-11	28/F	4.6488	ND	59.15
P-12	41/M	30.0645	7.6632	26.96
P-13	64/M	ND	25.3386	44.18
P-14	67/M	ND	10.4432	27.58

Note - The details of patients regarding age, mean ct values of ER α and ER β along with serum E2 values included in group III. ND, not detected

did not indicate an association between survival and expression of E2, ER α and ER β (Figure 4).

Discussion

An espalier of hormones governs the human body guiding it in each and every function. Any disturbance in the amount or expression of hormones can lead to major disruptions. One such hormone is Estrogen. It regulates not only reproductive processes but also cellular differentiation and growth. A harmonious relationship between the estrogen receptors (ER α and ER β) in tissue and estrogen derivatives like Estradiol, estriol, estrone and estretol in blood controls the function allocated to estrogen [8]. As like other parts of the body, even oral cavity is sensitive to changes in the levels of this hormone in blood and at receptor level [16]. The myraid of hormone related changes range from decreased salivation, feeling of dryness in oral cavity, atrophic mucosa, gingival inflammation or even desquamative gingivitis is reported

Table 3.4. Quantitative Data of Patients with Oral Squamous Cell Carcinoma

PATIENT NO.	AGE	ER α	ER β	E2
O-1	46/F	18.3374	ND	16
O-2	36/M	27.6897	10.3954	-
O-3	35/M	4.7371	11.4278	44.3
O-4	55/M	11.2148	24.9503	56.9
O-5	39/M	18.2476	ND	54.98
O-6	54/M	10.5406	ND	38.12
O-7	64/M	10.5406	ND	41
O-8	48/M	9.0917	ND	-
O-9	55/M	27.4714	22.8758	52
O-10	70/M	12.1604	10.4263	44
O-11	59/F	12.3488	ND	12
O-12	81/M	18.1611	ND	32
O-13	20/M	16.2488	ND	63.96
O-14	52/M	31.1637	ND	171.6
O-15	44/M	6.2623	24.9752	54.98
O-16	54/M	23.1405	ND	-
O-17	56/M	11.3798	ND	44.79
O-18	51/M	9.3326	ND	48.16
O-19	35/M	7.3984	ND	54.98
O-20	50/M	5.4467	ND	-

Note - The details of patients regarding age, mean ct values of ER α and ER β, grade of differentiation, along with serum E2 values included in group IV. ND, not detected

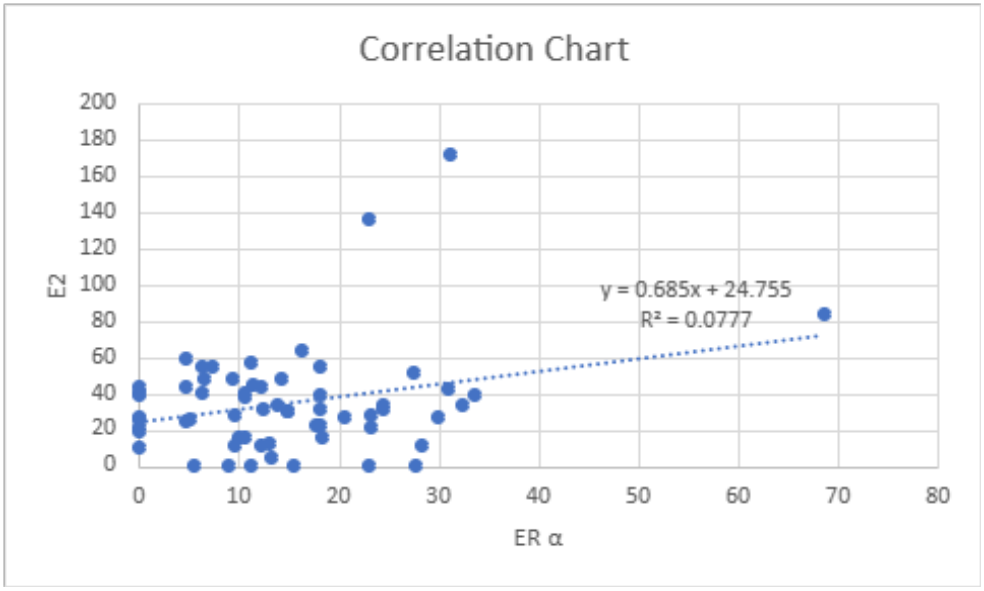

Figure 3. Graphical Presentation of Pearson Correlation Coefficient Representing PositiveCorrelation between Estradiol Levels and Quantitative Expression of ER α

Table 4. Analysis of Variance of Mean Estradiol Levels, ER α and ER β Levels in All Four Study Groups

Descriptives		N	Mean	95% Confidence Interval for Mean		P-value
				Lower Bound	Upper Bound	
Estradiol	1.00	10	22.5140	15.1561	29.8719	0.0327
	2.00	13	29.9077	23.1178	36.6975	
	3.00	13	41.2515	22.2367	60.2664	
	4.00	16	51.8606	33.2500	70.4713	
	Total	52	38.0765	30.3673	45.7858	
ER α ct mean	1.00	8	19.6827	13.4299	25.9356	0.3843
	2.00	11	18.2505	12.8780	23.6231	
	3.00	11	14.8156	7.9354	21.6958	
	4.00	20	14.5457	10.8995	18.1919	
	Total	50	16.2421	13.8566	18.6275	
ER β ct mean	1.00	6	18.7173	14.4714	22.9632	0.7841
	2.00	4	16.2595	0.1285	32.3904	
	3.00	4	19.7742	7.1755	32.3730	
	4.00	3	14.4817	-9.1290	38.0923	
	Total	17	17.6402	13.9558	21.3246	

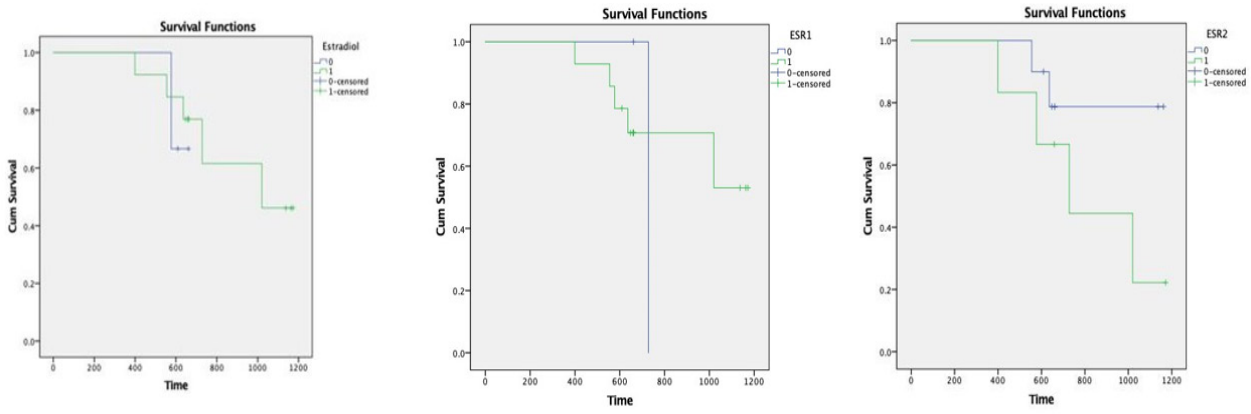

Figure 4. Kaplan-Meier Survival Analysis with 17 β- estradiol, Estrogen Receptor α (ERα) and Estrogen Receptor β(ER β) for survival analysis

in many post and pre-menopausal women which cured after treatment with hormone replacement therapy (HRT) [16], also it is found to influence the trajectory of events ranging from normal to dysplastic to oral squamous cell carcinoma [17].

If it is OSCC, then smokeless form of tobacco is considered to be the prime etiological factor constituting of nicotine which is known to induce various tumorigenesis pathways, catalyzed by metabolized products of Tobacco(nicotine). This list contains almost 14 polycyclic aromatic hydrocarbons (PAH) which can be introduced in tobacco and its related products by a curing process with exhaust gases (fire) before consumption and marketing [18]. Metabolization of these PAH is brought about by cytochrome P450 subfamily [19], consisting of CYP1B1, CYP1A1, CYP3A4 etc that catalyze the production of carcinogenic metabolites of both tobacco and estradiol(E2) [13]. The process of bioactivation of PAH by these enzymes results in formation of reactive metabolites which induces the formation of mutagenic DNA adducts which aid in carcinogenesis [19].

The results of E2 levels are in normal range but E2 levels did show 5% rise in levels in Group II, 13% rise in Group III and around 20 % rise in group IV as compared to group I. In this study, the levels of E2 were found significantly elevated in the group IV of OSCC. These results are in accordance with the study by Remenar et al who observed E2 predominance as compared to testosterone and dehydroepiandrosterone-sulfate in male HNSCC patients [13]. Almost 70% patients were males, who demonstrated statistically significant elevation in estradiol level as compared to control subjects. This proves that irrespective of the gender bias, the female sex hormone, estradiol can be raised in OSCC.

Bioavailable form of E2 falls in the range of 12.67 ±15.6 pg/ml for young, 10.9779±11.1 pg/ml for middle age to 10.2152±2.9 pg/ml for elderly males [20]. In women before menopause, the E2 values ranges from 15 to 350 pg/ml (depending upon hormonal cycles) and postmenopausal women should range below 10 pg/ml. In this study, Group I (normal control) reported a mean E2 level depicted the normal levels of subjects as females being in their follicular or ovulatory phase and males too in their reproductive ages [9]. The E2 levels in Group II are more than Group I because Group I depicted the results of healthy individuals without tobacco use. But Group II(Active control) demonstrated rise in mean E2 levels as compared to Group I(Normal control). The levels of E2 are inversely proportional to age, still we observed a rise in levels of E2. The only factor which was different from group I was that the subjects had the habit of Tobacco. E2 can also be produced by conversion of testosterone to E2 [21]. The conversion of testosterone is brought about by aromatase(CYP19 first member of cytochrome 450 family) [22], while tobacco is found to induce CYC1B1(third member of CYP1 family) [15, 22] that catalyzes the hydroxylation of estrone which can be converted into E2 [8]. CYP19 and CYC1B1 show similar affinity towards aromatase inhibitors [22]. CYP19 and CYC1B1 are close members of cytochrome 450 enzymes family, though present physiologically and the other may be a result of habit of tobacco, together might have contributed to the increased levels of E2. The levels of E2 showed an increase in spectrum in habit associated groups, maximum in OSCC group. In the present study, the estradiol levels were increased in group III of leukoplakia as compared to normal groups of I and II but less as compared to group IV of OSCC. These results were supported by the observations from the studies of Somers et al who observed that exposure to E2 caused the human samples to differentiate towards SCC in time dependent manner [23]. They stated that the exposure of tissue to E2 reduced the time from initiation of dedifferentiation to formation of actual tumor [23]. Shatalova et al observed that though the immunohistochemical staining for ERs was weak or absent in leukoplakic and control tissue, E2 was found to decrease apoptosis [15]. This suggests that irrespective of concentration of ERs, E2 can decrease apoptosis in premalignant cells, so increase in E2 levels may aid in progression of stage from leukoplakia to OSCC.

Numerous studies have identified the elevated levels of endogenous E2 in various carcinoma like cervical cancer [24], breast cancer [25, 26], endometrial cancer [27], uterine cancers [28] and also in other estrogen sensitive tissues were elevated levels favored tumor progression and invasion. Authors have also reported local production of E2 in the vicinity of malignant cells [21] .So the levels of E2 are highest in OSCC group because along with the habit of tobacco and role of cytochrome 450 enzyme, the carcinoma cells can locally produce E2. Estrogens stimulate mitosis, raising the mitotic rate and number of the epithelial cells in affected organ. The raised mitotic rate is a matter of concern as it facilitates increased chances of mutations being replicated before repair which may cause a surge in growth of early tumors [17]. Ishida et al also proved that treatment with ER antagonist, but not agonist, induced cell death(apoptosis) and also prevented adhesion of cultured oral SCC [14]. This signifies that the elevated levels of E2, as observed in present study, could affect the treatment protocol of OSCC and hence play a role in determining the prognosis of OSCC.

The cellular effects of E2 are mediated by Estrogen receptors. Extensive research has been done on the effect of E2 and ERs on almost every organ malignancy including breast, vagina, uterus, cervical, gastric, colon, endometrium, larynx, esophagus, prostate etc [29], but in OSCC, the role is still debated.

The observations demonstrated a statistically positive correlation between expression of ERα with levels of E2 indicating that the changes in ERα expression per unit changed the E2 value in positive direction according to Pearson correlation. It proved that the increase in levels of E2 were positively correlated with concentration of ERα receptor expression when observed in subjects of normal control to active control to premalignant to OSCC. But in this study, a dip in the ERα receptor expression was noted in the premalignant group of Leukoplakia as compared to Group I and II. Although due to the small sample size we cannot draw concrete conclusions, we theorize that the decreased concentration in expression of ER α in group III may be due to inverse correlation between of EGFR and ER α [6]. This can be further explained as E2 can

induce EGFR which is highly expressed in leukoplakic stages as compared to OSCC [30]. But EGF expression is less in leukoplakia as compared to OSCC which bind to EGFR when present in low concentrations in presence of serum and this assembly inhibit ER α expression [6]. These findings may justify the results of this study that the leukoplakic group showed increased levels of E2 in serum with reduced expression of ER α as compared to OSCC. And the levels of E2 are less raised in group I and II, so the results indicate a better expression of ER α in these two groups than the leukoplakic group.

The mean levels of ERα receptor expression further decreased in Group IV (OSCC). In line with the previous explanation, increase in tumor size is associated with increased EGF expression and EGFR is raised in established OSCC as compared to premalignancy [30-32]. This might have resulted in this decreased ERα receptor expression in OSCC. OSCC travels through a premalignant stage before actual invasion. These findings clearly indicate that Estradiol along with the habit of tobacco might induce molecular transformations in a cell which might accelerate its march towards malignancy.

There wasn't any appreciable correlation between levels of E2 and mean concentration of ER β expression when compared within group I, II and III and IV. We could not establish a pattern between the mean levels of ER β expression amongst the groups. Authors like Egloff et al and Doll et al do have reported no significant change in expression of ER β in OSCC tissue when compared with adjacent normal mucosa [10, 33] .

The observations of this study did not signify a possible correlation between survival and expression of ER α and ER β. These results are in accordance with the studies of Doll et al. [33, 34]. We also did not find an association between E2 levels and survival.

In conclusion, the levels of E2 can be used as a marker in predicting the progression of disease from normal tissue not exposed to tobacco to normal tissue exposed to tobacco to premalignant to OSCC. The ERα in tissue is positively correlated with the increased levels of E2 in serum, so ERα expression in tissue along with E2 in serum could be used to identify the subsets of patients with higher risk of developing OSCC. Especially the subset of subjects in group II (subjects with established tobacco habit but no appreciable change in oral mucosa) which had higher risk of developing the disease. The role of E2 in ER regulation can also be a subject of study for targeted therapies in OSCC which could help in improving patient's prognosis.

Author Contribution Statement

Data curation: Eesha Thakare. Supervision: Minal Chaudhary & Madhuri Gawande. Writing and editing: Eesha Thakare. Formal analysis: Eesha Thakare, Minal Chaudhary, Amol Gadbail, Prajakta Zade. Investigation: Eesha Thakare

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Ethical Statement

The study was approved by the Institutional Ethical Committee of DMIMSU, Nagpur.

Conflict of Interest

We the authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Data Availability Statement

http://hdl.handle.net/10603/490239. TheShodhganga@INFLIBNET Centre provides a platform for research students to deposit their Ph.D. theses. The manuscript has been read and approved by all the authors.

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