Chapter

Serum Sex Hormone Profiles in Potentially Resectable Esophageal Cancer

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Abstract

Esophageal cancer (EC) affects men far more commonly than women. Numerous epidemiological studies have suggested that the hormonal milieu may play a role in this gender bias. However, there is little known about circulating sex hormone levels in relation to the risk of EC development. In this chapter, the correlation between circulating sex hormone levels and mRNA expression of estrogen receptors (ER) in normal esophageal mucosal samples and EC biopsies from patients with potentially resectable EC is studied. Moreover, the association of serum sex hormones levels with and clinico-pathological features of EC is analysed.

Keywords: esophageal, cancer, squamous, adenocarcinoma, sex hormones, estrogen, testosterone, estradiol

1. Introduction

Sex steroid hormones are essential for normal reproductive health in both sexes. Estrogens in women and androgens in men are crucial for development of sexual organs and regulation of gametogenesis. They also play vital roles in regulating physiological functions of other non-target tissues and organs. For instance, estrogens are involved in the maintenance of bone mass, regulation of lipoprotein synthesis, prevention of urogenital atrophy, regulation of insulin responsiveness, and maintenance of cognitive function.

Sex steroids hormones are also linked with development, progression, or treatment of several cancers that include breast, uterine, prostate, and testicular cancer [1].

Indeed, the correlation between endogenous sex steroid hormones and the risk of developing breast cancer is well described in the literature. Besides, the use of anti-estrogenic therapy is associated with better local control and survival outcome in patients with breast cancer [2].

Esophageal cancer (EC) is the eighth most common cancer and the sixth most common cause of cancer mortality worldwide [3]. Despite developments in treatment modalities, estimated overall five-year survival rate for patients with EC is still poor, one of the characteristic features of EC, especially esophageal adenocarcinoma (EAC) is a persistence gender bias over several decades, in all races and across the world [3–5]. Although EC is not a hormone-dependant tumour, several epidemiological studies suggested that female sex hormones may have a protective role against

the development of this aggressive malignancy [6, 7]. There is mounting evidence that the male predominance associated with oesophageal cancer is age dependent. The gender ratio is at its highest at a younger age, whereas the incidence difference between older men and women is smaller afterwards [8, 9]. This gender ratio seems to have been consistent during the last decades, despite the increasing incidence in EAC.

Several epidemiological studies investigated the possible association between the endogenous and exogenous sex hormonal exposure and EC. Lindblad et al. suggested that the use of HRT was not associated with a reduced risk of EC of any histological type [10]. Similarly, two recent studies examined the association between the use of HRT and the risk of EAC and squamous cell carcinoma (ESCC) in postmenopausal women [11, 12]. Freedman et al. found that the use of HRT was significantly associated with lower risk of ESCC and with a non-significant lower risk of EAC. Moreover, older age at menopause was inversely associated with ESCC. This risk reduction was more evident in women with intact uteri and who received estrogen and progestin containing HRT. Therefore, it was suggested that higher estrogen and progesterone levels may be related inversely to EC and in this way help explain the lower incidence rates in women compared to men [11]. In contrast, another study of 161,086 postmenopausal women involved in the Women's Health Initiative (WHI), showed that the risk of ESCC was only lower among HRT users with a decreased risk mainly among current users of estrogen and progestin containing HRT. No association was observed between the use of HRT and the risk of EAC. Also, no other reproductive or hormonal factors were significantly associated with the risk of either ESCC or EAC [12].

A nested case–control study within a prospective UK cohort and meta-analysis found that women prescribed HRT had a reduced risk of OC (adjusted RR for HRT versus no HRT prescriptions, 0.68, 95% CI 0.53–0.88; $p < 0.004$) [13]. There were no significant differences in cancer risk by HRT type, estimated duration of HRT use or between past and current users [13]. Recently, the Million Women Study (MWS) by Green et al examined risk of esophageal and gastric cancers in relation to reproductive factors in a large UK cohort [14]. They have shown that risks of both esophageal and gastric cancers were higher in postmenopausal than in pre- or peri-menopausal women, and, among postmenopausal women, risks were higher the younger the women were at menopause [14]. In contrast to some studies where no association of childbearing on the risk of AEC was identified [15], the MWS demonstrated that the association between parity and risk of EC was more significant for ESCC than for EAC and age at menarche was significantly associated with EAC but not ESCC [14]. Green et al therefore suggested that the reduced risks associated with menopausal status and with hormone therapy use are consistent with a hypothesis that exposure to estrogens reduces the risk of EC [14].

On the other hand, in a large population-based cohort of 87, 323 postmenopausal women with breast cancer, the potential effects of tamoxifen (Selective ER modulator mostly acts as an anti-estrogen) used for breast cancer treatment was evaluated. In this study, there was no increase in the risk of developing EC in breast cancer patients who received adjuvant tamoxifen treatment in comparison to the control group [16, 17]. However, a different large population-based study of 138,885 women (by Chandanos et al.) suggested a 60% risk increase of EAC among the exposed group but did not achieve statistical significance. In contrast, there was an increased risk of ESCC and lung cancer observed only in the unexposed cohort, indicating that the confounding factor of smoking might explain the increased incidence during the unexposed period [18].

Interestingly, a dose-dependent risk reduction in EC by breastfeeding was suggested [19]. In a recent study, based on pooled data from several large case–control studies, endogenous reproductive factors and exogenous factors were evaluated in women. Breastfeeding was associated dose-dependently with a reduced risk of

EAC, while parity, menstruation, history of pregnancy, use of oral contraceptives or of HRT were not associated with the risk of developing this tumour [20, 21].

In a cohort of patients with prostate cancer, a reduced risk of EAC but not of ESCC was noted. Hence, it was suggested that a diagnosis of prostate cancer may be linked with aetiological factors that are negatively associated with EAC, or anti-androgen therapy may influence the development of EAC [22]. In contrast, estrogen exposure in a national cohort of men with prostate cancer did not show any reduced risk of a second AEC [23]. Despite these efforts to explain the relation between female reproductive factors and risk of development of EC, the results are rather unclear and contradict each other. This could be partially explained by the fact that the number of women with EC included in those studies was relatively small, which could have the affected potential significance of the results [24].

Recently, it has suggested that the ER system is involved in EC progression and thus may provide a novel target for the treatment of EC [25]. However, there is little known about sex hormones levels in relation to the risk of EC development. In this study we aimed to:

1. Describe sex hormones profiles in a cohort of patients with established OC.

2. Identify whether there is any correlation between circulating sex hormone levels and mRNA expression of estrogen receptors (ER) in normal esophageal mucosal samples and EC biopsies.

3. Analyse the correlation between circulating sex hormone levels and clinic-pathological features of EC.

2. Material and methods

2.1 Patient cohort

Joint ethical approval for the research protocol was acquired from the Derbyshire Research Ethics Committee and Derbyshire Hospitals Research and Development office. Written, informed consent was obtained from all patients included in this study. EC samples and matched normal tissue taken from adjacent macroscopic mucosa from the same patient were collected from resected EC specimens of 34 patients [EAC: n = 28; ESCC: n= 6] who underwent esophagectomy. Normal samples were microscopically examined by a consultant pathologist to confirm normal features.

2.2 mRNA analysis by qRT-PCR

Total RNA was extracted from tissue samples (30 mg), ground in liquid N_2 with a pestle and mortar and from cell lines (10^4 cells) using the RNeasy Mini kit method (QIAGEN, UK) as per manufacturer's protocol. 300 ng of total RNA was reverse transcribed with (+RT) or without (-RT) reverse transcriptase (RT) using the high-capacity cDNA reverse transcription kit (Life Technologies, Paisley, UK). 2 μl of cDNA were amplified by real time PCR with commercially available TaqMan assays (Life Technologies, Paisley, UK) for *ESR1* (Hs00174860_m1), *ESR2* (Hs01100353_m1), and the reference genes *GAPDH* (Hs02758991_g1), *PGK1* (Hs00943178_g1), and *ACTB* (Hs01060665_g1) in a Chromo 4 thermal cycler (Bio-Rad Laboratories LTD, Hemel Hempstead, UK). Expression of *ESR1* and *ESR2* was quantified relative to the geometric mean of three reference genes and reported as relative to max using the GenEX software Version 5 (MultiD, DE) in accordance with MIQE guidelines [26].

2.3 Immunohistochemistry

Immunohistochemistry (IHC) slides were prepared in the Histopathology Department at the Royal Derby Hospital. Normal mucosa and EC samples were stained using ERα and ERβ antibodies (NCL-L-ER-6F11 and 6007907, respectively, Novacastra, Newcastle, UK). ERα and ERβ positive breast cancer samples were used as positive controls. The 'H-score method was used to measure the strength of ER-staining in normal esophageal mucosa and matched tumour samples [27]. Positive staining was defined as an H-score ≥ 10 in this study.

2.4 Blood samples

Blood samples were collected from 34 patients with histologically proven EC. Once the patient was anaesthetised, 10 ml of central venous blood was taken using gold-top Vacutainer Serum Separation Tubes (BD Vacutainer® SST™). Blood samples were immediately transported to the Clinical Chemistry laboratory in Royal Derby Hospital for analysis. Fasting serum level of testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), 17-β oestradiol (E2) were measured using *ElectroChemiLuminescence ImmunoAssay* (ECLIA, Roche) and as per manufacturer's protocols. *Elecsys and Cobas e immunoassay 411 analyzer* was used for running of ECLIA (Roche-diagnostic, USA). The free androgen index (FAI) was calculated as the total testosterone to SHBG ratio [28].

2.5 Statistical analysis

For qRT-PCR on primary tissues, the two-tailed Wilcoxon signed rank test was used for matched cases while the two-tailed Mann-Whitney *U* test was used for non-matched variables. Either the two-tailed Mann-Whitney *U* test or Kruskal-Wallis test, as appropriate, was used to establish relationships between hormone levels, ER mRNA and clinico-pathological features. Correlation coefficient (r) was calculated using two-tail Spearman correlation. Statistical differences were calculated using SPSS Statistics® for Windows™ v21 software from IBM SPSS Statistics (Feltham, UK) and GraphPad Prism® v6 (La Jolla, CA, USA). A value of $p \leq 0.05$ was considered as statistically significant.

3. Results

3.1 Clinico-pathological characteristics of recruited patients

The clinico-pathological characteristics of EC patients included in the analysis are summarised in **Table 1**. Median age was 65 years (range, 30 – 79 years). There were 28 males and 6 females with male-to-female ratio of 5.7:1. Of those 34 patients, 28 patients had EAC and 6 patients had ESCC. One-year disease specific survival was approximately 73.5%. Twenty-five (74%) patients received neoadjuvant therapy.

3.2 Sex hormones profiles

The median (Inter quartile range) (IQR) of the serum sex hormones levels (34 patients) against their corresponding laboratory reference ranges (Derby Hospital biochemistry department) is summarised in **Table 2**. Median serum levels of FSH and LH hormone were higher than normal. However, the median of serum levels of E2, progesterone, testosterone, and SHBG were within the normal ranges **Table 2**.

Patients recruited		**34**
Median age (years)		65 (range, 30-79)
One-year disease-specific survival		73.5%
Gender	Male	28 (83%)
	Female	6 (17%)
Histology	EAC	28 (76%)
	ESCC	6 (24%)
Tumour depth (T-stage)	T1	8 (24%)
	T2	3 (9%)
	T3	23 (67%)
Nodal involvement	No (N0)	15 (44%)
	Yes (N1)	19 (56%)
Tumor differentiation	Moderate	25 (74%)
	Poor	9 (26%)
Vascular invasion	No	21(62%)
	Yes	13 (38%)
Barrett's Metaplasia	No	13 (46%)
	Yes	15 (54%)
Circumferential resection margin	Not involved	23 (68%)
	Involved	11 (32%)
Preoperative chemotherapy	No	9 (26%)
	Yes	25 (74%)

Table 1.
Patient Characteristics.

In a gender-based analysis, there were significant higher median (IQR) levels of LH and FSH in female patients compared to LH and FSH levels in male patients. In contrast, women had significantly lower median levels E2, testosterone compared to men. There was no significant difference in serum levels of progesterone and SHBG between men and women (**Table 3**).

3.3 Correlation between serum sex hormones level and estrogen receptor expression

The results of correlation between serum sex hormones and ERα and ERβ expression at mRNA are summarised in **Tables 4** and **5**. There was no significant correlation between the expression of ERα or ERβ mRNA in normal mucosa or tumour samples and serum level of LH, FSH, E2, Testosterone, FAI, or SHBG. In analysis of the correlation of hormones levels with estrogen receptors expression in both genders, we found that there was significant inverse correlation between testosterone level and ERβ mRNA expression in normal mucosa from male patients ($r = -0.41$, $p = 0.03$) (**Table 6**). In female patients, there was significant inverse correlation between progesterone level and ERα mRNA expression in EC samples ($r = -0.87$, $p = 0.04$) level (**Table 7**). No correlation was demonstrated between the levels of rest of sex hormones and ERs expression in either gender (**Tables 6** and **7**).

Hormone (reference range)	Median (IQR) of serum level
LH (1.7 – 8.6 IU/L)	9.9 (7, 17) IU/L
FSH (1.5 – 12.4 IU/L)	17 (9.6, 28) IU/L
E2 (28 – 167 pmol/L)	73 (50, 95) pmol/L
Progesterone (0.7 – 4.3 nmol/L)	1.1 (0.5, 1.15) nmol/L
Testosterone (8.3 – 27.8 nmol/L)	12 (6.5, 16.5) nmol/L
SHBG (14.5 – 48.4 nmol/L)	52 (32, 68) nmol/L
FAI (34 – 106 %)	20.5% (12%, 31%)

LH, luteinizing hormone; FSH, follicular stimulating hormone; E2, 17β-Oestradiol; SHBG, serum hormone binding globulin; FHI, free androgen index.

Table 2.
The median (IQR) of serum sex hormones levels of patients with EC (n = 34).

Hormone (reference range)	Median (IQR) of serum level		
	Male (n = 28)	Female (n = 6)	*p* value*
LH (1.7 – 8.6 IU/L)	9.5 (6.5 – 12) IU/L	33.5 (21 – 45.5) IU/L	0.004
FSH (1.5 – 12.4 IU/L)	13 (7.8 - 23) IU/L	62 (40 – 90) IU/L	0.001
E2 (28 – 167 pmol/L)	78 (59 – 98) pmol/L	50 (35 – 50) pmol/L	0.002
Progesterone (0.7 – 4.3 nmol/L)	1.2 (0.8 – 2.4) nmol/L	0.9 (0.8 – 1.5) nmol/L	0.51
Testosterone (8.3 – 27.8 nmol/L)	14 (8 – 20) nmol/L	0.5 (0.3 – 1.1) nmol/L	0.0001
SHBG (14.5 – 48.4 nmol/L)	54 (32 – 65) nmol/L	40 (28.5 – 89) nmol/L	0.84
FAI (34 – 106 %)	21.5% (16% – 35%)	1% (0.5% – 3%)	0.001

LH, luteinizing hormone; FSH, follicular stimulating hormone; E2, 17β-Oestradiol; SHBG, serum hormone binding globulin; FHI, free androgen index.
**Calculated using two-tail Mann Whitney U test.*

Table 3.
Gender-based comparison of serum sex hormones levels in patients with EC.

Hormone	ESR1 (ERα) mRNA expression			
	Normal mucosa		Tumour	
	r	p-value*	r	p-value*
LH	0.08	0.69	0.16	0.41
FSH	- 0.16	0.38	0.04	0.83
E2	0.11	0.55	0.03	0.99
Progesterone	0.2	0.26	0.22	0.2
Testosterone	0.10	0.56	0.10	0.57
SHBG	- 0.2	0.32	0.08	0.68
FAI	0.31	0.11	- 0.08	0.7

LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, 17β-Oestradiol; SHBG, serum hormone binding globulin; FHI, free androgen index; r, the correlation coefficient.
**Calculated using two-tail Spearman correlation.*

Table 4.
Correlation between serum sex hormones levels and ERα mRNA expression.

Hormone	ESR2 (ERβ) mRNA expression			
	Normal mucosa		Tumour	
	r	p-value*	r	p-value*
LH	- 0.22	0.24	- 0.01	0.94
FSH	0.08	0.67	- 0.03	0.85
E2	0.02	0.92	- 0.16	0.38
Progesterone	0.08	0.66	- 0.18	0.33
Testosterone	- 0.03	0.08	- 0.14	0.43
SHBG	0.07	0.7	0	1
FAI	- 0.14	0.49	- 0.25	0.21

LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, 17β-Oestradiol; SHBG, serum hormone binding globulin; FHI, free androgen index; r, the correlation coefficient.
**Calculated using two-tail Spearman correlation.*

Table 5.
Correlation between serum sex hormones levels and ERβ mRNA expression.

Hormone	ESR1 (ERα) mRNA expression				ESR1 (ERβ) mRNA expression			
	Normal mucosa		Tumour		Normal mucosa		Tumour	
	r	p-value*	r	p-value*	r	p-value*	r	p-value*
LH	0.13	0.53	0.19	0.36	- 0.26	0.21	- 0.06	0.77
FSH	- 0.13	0.52	- 0.04	0.85	0.07	0.72	- 0.17	0.41
E2	0.07	0.73	0.08	0.69	0.05	0.80	- 0.07	0.73
Progesterone	- 0.13	0.54	0.08	0.71	- 0.27	0.19	0.10	0.62
Testosterone	0.09	0.64	0.26	0.19	- 0.41	0.03	- 0.03	0.88
SHBG	0.13	0.52	0.08	0.69	- 0.01	0.99	- 0.07	0.74
FAI	0.35	0.11	0.09	0.68	- 0.22	0.36	- 0.14	0.53

LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, 17β-Oestradiol; SHBG, serum hormone binding globulin; FHI, free androgen index; r, the correlation coefficient.
**Calculated using two-tail Spearman correlation.*

Table 6.
Correlation between serum sex hormones levels and estrogen receptors mRNA expression in male patients (n = 28).

Hormone	ESR1 (ERα) mRNA expression				ESR1 (ERβ) mRNA expression			
	Normal mucosa		Tumour		Normal mucosa		Tumour	
	r	p-value*	r	p-value*	r	p-value*	r	p-value*
LH	- 0.80	0.20	- 0.80	0.20	- 0.40	0.60	0.40	0.60
FSH	- 0.36	0.55	-0.22	0.74	0.10	0.87	0.31	0.61
E2	0.71	0.18	0.71	0.18	- 0.35	0.56	0.35	0.56
Progesterone	- 0.62	0.27	- 0.87	0.04	- 0.21	0.74	0.36	0.55
Testosterone	0	1.0	- 0.20	0.75	0.60	0.29	- 0.70	0.19
SHBG	- 0.56	0.32	- 0.15	0.81	0.46	0.43	- 0.21	0.74
FAI	0.37	0.54	0	1.00	0	1.00	- 0.16	0.80

LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, 17β-Oestradiol; SHBG, serum hormone binding globulin; FHI, free androgen index; r, the correlation coefficient.
**Calculated using two-tail Spearman correlation.*

Table 7.
Correlation between serum sex hormones levels and estrogen receptors mRNA expression in female patients (n = 6).

Hormone	Disease specific one-year survival			
	Males		Females	
LH	R	- 0.33	R	0.26
	p-value[*]	0.10	*p-value*[*]	0.74
FSH	R	0.12	r	0.36
	p-value[*]	0.56	*p-value*[*]	0.55
E2	R	0.32	r	1.000[**]
	p-value[*]	0.10	*p-value*[*]	< 0.0001
Progesterone	R	0.16	R	0.36
	p-value[*]	0.46	*p-value*[*]	0.55
Testosterone	R	0.19	R	0.35
	p-value[*]	0.37	*p-value*[*]	0.56
SHBG	R	0.21	R	0.36
	p-value[*]	0.29	*p-value*[*]	0.55
FAI	r	0.21	r	0.19
	p-value[*]	0.35	*p-value*[*]	0.76

LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, 17β-Oestradiol; SHBG, serum hormone binding globulin; FHI, free androgen index; r, the correlation coefficient.
[*]Calculated using two-tail Spearman correlation.

Table 8.
Correlation of serum sex hormones level & 1-year disease specific survival.

Hormone		Histology (EAC vs ESSC)	T-Stage	LN-stage	Grade	VI	BM	CRM
LH	r	0.04	- 0.08	0.21	0.18	0.04	- 0.01	- 0.06
	p-value[*]	0.87	0.71	0.30	0.39	0.83	0.98	0.78
FSH	r	- 0.03	- 0.18	0.25	- 0.13	- 0.28	- 0.02	- 0.16
	p-value[*]	0.88	0.36	0.21	0.61	0.16	0.943	0.50
E2	r	- 0.27	- 0.125	0.12	0	0.10	-.018	0.02
	p-value[*]	0.17	0.53	0.54	1.00	0.61	0.93	0.92
Progesterone	r	- 0.25	- 0.21	0.39	0.27	- 0.16	- 0.06	- 0.25
	p-value[*]	0.28	0.32	0.05	0.19	0.46	0.77	0.23
Testosterone	r	0.21	0.18	0.15	0.12	0.06	- 0.04	0.05
	p-value[*]	0.28	0.35	0.44	0.53	0.78	0.86	0.81
SHBG	r	0.24	0.13	0.08	0.13	- 0.09	- 0.17	- 0.01
	p-value[*]	0.24	0.51	0.69	0.53	0.65	0.39	0.96
FAI	r	- 0.17	- 0.08	- 0.24	- 0.22	- 0.15	0.20	- 0.50
	p-value[*]	0.61	0.72	0.29	0.33	0.61	0.37	0.02

LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, 17β-Oestradiol; SHBG, serum hormone binding globulin; FHI, free androgen index; T-stage, tumour depth; LN-stage, lymph node involvement; VI, vascular invasion; BM, barrett's metaplasia; CRM, circumferential resection margin; r, the correlation coefficient.
**Calculated using two-tail Spearman correlation.*

Table 9.
Correlation between serum sex hormones levels and pathological features in male patients (n = 28).

Hormone		Histology (AEC vs ESSC)	T-Stage	LN-stage	Grade	VI	BM	CRM
LH	r	- 0.78	- 0.78	0.78	- 0.26	0.78	- 0.78	0
	p-value[*]	0.23	0.23	0.23	0.74	0.23	0.23	1.00
FSH	r	- 0.18	- 0.74	0.74	0.18	0	-.725	- 0.15
	p-value[*]	0.77	0.15	0.15	0.77	1.00	0.17	0.81
E2	r	- 0.25	- 0.41	0.41	0.25	- 0.41	0.25	- 0.61
	p-value[*]	0.69	0.50	0.50	0.69	0.50	0.69	0.27
Progesterone	r	- 0.73	- 0.44	0.44	- 0.18	0.74	- 0.73	0.15
	p-value[*]	0.17	0.45	0.45	0.77	0.15	0.17	0.81
Testosterone	r	0	0.58	- 0.58	0.71	0.87	- 0.35	0.87
	p-value[*]	1.00	0.31	0.31	0.18	0.06	0.56	0.06
SHBG	r	0.73	0	0	- 0.18	- 0.44	- 0.18	0.15
	p-value[*]	0.17	1.00	1.00	0.77	0.45	0.77	0.81
FAI	R	0.56	0.15	- 0.15	0.56	0.76	- 0.19	0.30
	p-value[*]	0.33	0.81	0.81	0.33	0.14	0.76	0.62

LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, 17β-Oestradiol; SHBG, serum hormone binding globulin; FHI, free androgen index; T-stage, tumour depth; LN-stage, lymph node involvement; VI, vascular invasion; BM, barrett's metaplasia; CRM, circumferential resection margin; r, the correlation coefficient.
**Calculated using two-tail Spearman correlation.*

Table 10.
Correlation between serum sex hormones levels and pathological features in female patients (n = 6).

3.4 Correlation between serum sex hormones levels and clinico-pathological features

Gender-based analysis of correlation between serum hormones levels and survival outcome at one year and pathological features after intended curative resection was performed using two-tail Spearman test. There was highly significant positive correlation between 1-year disease survival outcome and serum E2 level in female patients ($r = 1$, $p < 0.0001$) (**Table 8**). However, no significant association was determined between sex hormones levels and 1-year disease specific survival in male patients (**Table 7**).

No correlation was demonstrated between serum hormonal levels and pathological features of EC from either male or female patients (**Tables 9** and **10**).

4. Discussion

In this study, we investigated sex steroid hormones profiles in patients with established EC. Our results demonstrated that there was no correlation between serum level of LH, FHS, E2, SHBG, or FAI and ER expression in normal esophageal mucosa or EC from both genders. Whilst 1-year disease survival is significantly correlated with high E2 level in female patients, no significant correlation is identified between survival outcome and sex hormones level in male patients.

The disparity in the incidence of EC between males and females has always raised the question regarding the role of sex hormones and sex EC in esophageal carcinogenesis. In an *in vitro* study, Matsuoka et al. were first to investigate the

effect of E2 and testosterone on the growth of newly established cancer cell lines of ESCC origin, which expressed both ER and androgen receptor (AR). They found that the growth rate of the cell lines was inhibited by estrogen and enhanced by testosterone [29]. In a similar way, Utsumi et al. suggested that the presence of ER arbitrates the inhibitory effect of estrogen when it was noted that the growth of ER positive transplanted tumour was significantly greater in male than it was in female nude mice. Such a difference was not observed for ER negative tumour. Also, the growth rate of ER positive tumours was enhanced in oophorectomised female mice and significantly suppressed with a physiological dose of E2 compared to ER negative cancer [30, 31]. Likewise, Ueo et al studied sex hormone dependency and hormone responsiveness on both ER positive (KSE-1) and ER negative (KSE-2) ESCC cell lines transplanted in male and female nude mice. They found that the administration of E2 significantly inhibited the growth of KSE-1 tumours in both males and females in conjunction with an increase in E2 levels. No similar influence on KSE-2 growth was identified. Therefore, they suggested that the growth of human EC cells with sex hormone receptor is influenced by circulating hormone levels and can be manipulated by systemic E2 administration [32].

EAC often expresses ER, which suggests a possible biological involvement of steroid hormones. Wang et al. suggested that ER and their signalling could have a role in EC development when he found that 43.75% (21/48) and 20.83% (10/48) of EC cases were positive for ER and progesterone receptor (PR) respectively, while these receptors were negative in esophageal tissues taken from normal subjects [33]. Similarly, Tiffin et al suggested that the role of ER may warrant further investigation when they identified mild to moderate ER staining in most of their esophageal tissue samples. However, the author did not discriminate between the ER subtypes detected [34]. Interestingly, Nozoe et al found that ERα expression was significantly higher in male patients with ESCC in comparison to females. Also, a positive expression of ERα plus negative expression of ERβ was found to be an unfavourable independent prognostic indicator in patients with ESCC. Therefore, they suggested that hormonal therapy using estrogen may have a role in improving the outcome in this type of cancer and its possible anti-tumour effect requires more in depth both laboratory and clinical based investigations [35]. Akgun et al. studied the expression of ERβ in Barrett's metaplasia and associated EAC. They concluded that all EAC and most precursor lesions, Barrett's metaplasia with or without dysplasia, express ERβ in a significantly high percentage of the cells. These findings raise the possibility that EAC may benefit from treatment and/or chemoprevention by SERM [36]. Likewise, Liu et al. found that ERβ1, ERβ2, ERβ3 and ERβ5 are overexpressed in EAC compared to its precursor lesion Barrett's metaplasia negative for dysplasia, suggesting a significant biological role [37]. In contrast, Kalayarasan et al. studied the expression of ERα and ERβ as well as Progesterone Receptors (PR) in both tumour tissue and adjacent normal mucosa samples of 45 cases of EC (ESCC = 30 cases, EAC= 15 cases). They found neither tumours nor normal mucosa expressed ERα or PR. However, all cases of EAC, irrespective of their stage/grade, were strongly positive for ERβ and the intensity of staining in tumours was significantly higher than in normal adjacent mucosa. Therefore, they suggested that estrogen may have an effect on the growth of EAC and this effect may be mediated by ERβ [38]. Despite numerous studies assessing the role of sex hormones in AEC development, the findings are still featured by some inconsistency. It seems therefore important that the molecular mechanisms of ER in esophageal carcinogenesis are further clarified.

Recently, Zuguchi et al found that there is increased nuclear ERβ reactivity in human ESCC in comparison to matched normal mucosa [39]. Moreover, increased ERβ expression seems to have unfavourable correlation to the histo-pathological stage of the disease [39]. Therefore, they concluded that EC is an estrogen dependant

malignancy and ERβ might provide an additional therapeutic target for treatment of ESCC [39]. Another study by Sukocheva et al demonstrated that tamoxifen and raloxifen inhibited the growth of esophageal AC cell lines proliferation by inducing apoptosis and cell cycle arrest [40]. However, the study did not differentiate between the role of each receptor, especially when we know for fact that SERMs have different agonist and antagonist function of ER in different body tissues.

Several studies demonstrated that women undergoing curative EC resection have better long term survival outcome compared to men [6, 7]. In a case control study, Wang et al, found that there was a low serum E2 level from healthy controls from an area with a high incidence of ESCC compared to counterparts from a low incidence area in China [41]. Hence, they suggested that the discrepancy in incidence of ESCC may be explained by the lack of a protective E2 effect [41]. In another study, Petrick JL et al identified a high ratio of androgens to estrogens – particularly testosterone:estradiol ratio – was more common in EAC patients than controls, including after restriction to cases without weight loss in the previous 5 years [42]. The lack of association between circulating E2 and ER expression in this study may reflect the theoretical possibility that ER expression is influenced more by intra-tumor E2 rather than circulating hormone concentrations [43, 44]. For instance, Recchione et el found that E2 level in breast cancer samples is significantly higher compared to serum level of E2. Similarly, in a comparison of blood and breast cancer tissue concentrations sex steroids by Secreto et al showed that there was significantly higher level of E2 in the tumours than in the blood [45].

Recently, Xie, S-H et al found that an increased disease-specific mortality with lower SHBG levels and higher FSH levels in male EAC patients without surgical treatment. No clear associations were observed for dehydroepiandrosterone sulphate, LH, prolactin, testosterone, 17-OH-progesterone, progesterone, E2, androstenedione, testosterone:estradiol ratio or free testosterone index [46]. In our study, there was no correlation between sex hormones level and pathological features. However, we found higher serum E2 level had significant positive correlation with 1-year disease specific survival in women. This may indicate that E2 may play antiproliferative effect and in turn reduce the risk of developing distant micro metastases. For example, high dose estrogens, like diethylstilbestrol found to paradoxically inhibit breast cancer growth by activation of Fas/FasL apoptotic pathway [47]. In another in vitro study, Al-Khyatt et al found that ERs antagonists induced apoptosis in EC cell lines proliferation. Thus, they concluded that their findings may indicate that the ER system is involved in OC progression and thus may provide a novel target for the treatment of OC [25].

In a study by Awan et al [48], a raised testosterone level had a positive correlation with the presence of EC compared to control group. Furthermore, serum testosterone levels decreased after surgical resection of the tumour [48]. In this study it was demonstrated that testosterone had an inverse correlation with ERβ mRNA expression in normal mucosa from male patients. Several studies investigated the role of androgens in the pathogenesis of estrogen-dependant cancers like breast cancer [49]. Nevertheless, the significance of cross-talk between androgens and ER in EC is not clear and warrants further investigation [50].

The number of patients used in this study is relatively small, especially female patients. This could be the reason behind the lack of any association between serum sex hormones and ER expression or pathological features. Another limitation is there was no matched healthy control. The serum sex hormones levels of healthy controls could have been used for comparison as well as studying the correlation between hormones levels and risk factors profiles for EC.

In summary, in this study, there was no association between sex hormones profiles and the expression of ER or pathological features of EC in both genders.

Interestingly, survival outcome was better in women with increased serum level of E2. Current published evidence supports that sex hormones may play a role in esophageal carcinogenesis. Hence, future research work may include a population-based study looking into the correlation of sex hormones and risk factors profiles for EC in healthy volunteers and patients is required. Likewise, a well-designed *in vivo* study to address the potential therapeutic role of ERα and ERβ in treatment of EC is warranted.

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