

Asian Journal of
Biological
Sciences



Research Article

Clinical Analysis of Elevated Free Oestradiol in Men of Eket: An Insight into Preventing Prostate Proliferation

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Abstract

Background and Objectives: Diagnosing the onset of prostate cancer before the manifestation of symptoms is the challenge in detecting diseases. For intervention on the risk of cancer, this investigation assayed the *in vitro* estrogen elevation in men's population, by quantifying free Oestradiol and sex hormone-binding globulin level of selected men in Eket-Nigeria. **Materials and Methods:** A 197 men's subjects were selected using a simplified sample size formula. Blood serum samples were prepared from the blood collected from the men's donors. The assay was carried out using estrogen (E2) and sex hormone binding globulin (SHBG), enzymes linked immunosorbent assay (ELISA) technique. The ratio of SHBG to E2 was calculated to get free oestradiol. **Results:** A 28 of 197 men have elevated E2 beyond the recommended reference range 15-60 pg mL⁻¹. This accounted for 14.21% of 28/197 men with elevated free estrogen. **Conclusion:** Comparing these figures with our control reference community where there was a low level of E2, one can infer that the men of Eket pose a risk of prostate cancer due to elevated E2. This implied that elevated testosterone is aromatized to estrogen by aromatase. Therefore, men of Eket should receive anti-estrogen therapy to reduce the risk of prostate cancer.

Key words: Perceived risk, cancer, epidemiology, E2-SHBG, prostate

Citation: Victor Eshu Okpashi, Bayim Peter-Robert Bayim and Magdalene Obi-Abang, 2020. Clinical analysis of elevated free oestradiol in men of Eket: An insight into preventing prostate proliferation. Asian J. Biol. Sci., 13: 9-15.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Estrogen plays a role in prostate disease, premalignant dysplasia usually occurs when a rising amount of estrogens are induced in combination with increasing doses of androgens. Uncontrolled growth of body cell at the earliest stage is an indication of cancer¹. In many parts of the human or animal, cancer cells can grow, dispersed to other parts of the body. The prostate is a male gland; it is located below the bladder and in front of the rectum. The size of a prostate is about the size of a walnut in younger men, the size usually grows bigger in older men. The seminal vesicles which produce and secrete the fluid for semen are located behind the prostate. The transfer of urine and semen out of the body is done by the urethra through the penis. This urethra passes through the center of the prostate. Several investigations on the hormone that triggered the onset of prostate cancer are directed on the functions of the male sex hormone-androgens². However, Oestradiol is implicated as a causative vehicle in the etiology of prostate cancer proliferation. Links between the risk of prostate cancer and prediagnostic serum estrogen levels were found in some epidemiological studies³. There are shreds of evidence which suggests that E2 is involved in carcinogenesis of the prostate gland. For instance, the genes for polymorphisms which were found in the E2 metabolism pathway were tandem with prostate carcinoma risk⁴. According to suggestions, a polymorphism in codon 10 of estrogen receptor alpha might serve as a risk factor for prostate cancer proliferation⁵. Cancel-Tassin *et al.*⁶ report that the variants in GGGA-polymorphism gene which originate from the estrogen receptor alpha gene may exacerbate the risk of developing prostate cancer⁶. The use of E2 in the treatment of castration-resistant cancer contradicted other works some shreds of evidence suggested that prostate cancer was caused by elevation or a rising E2. It is on this premise that International Agency for Research on Cancer (IARC) classified estradiol-17 β a carcinogen⁷⁻⁸. In

humans, they are reciprocal evidence which shows that E2 affects the prostate gland which makes the medium conducive enough for cancer onset. But the circulating E2 may not complement well with the intra-prostatic stage. Especially, if it is consider that the corroboration of intra-prostatic production of E2 is not well established. This also elucidates the values of serum E2 which offers an accessible surrogate marker that established the linkage between E2 and prostate cancer progression⁹. On the epidemiological perspective, data from adult-men have shown mixed results, where one finds a link between rising plasma E2 with the potential risk of eliciting prostate cancer¹⁰ and another correlating the risk with chronically elevated estrogens¹¹. There are questions about the protective effect of androgens against high-grade CaP [prostate cancer]. This showed that 17 β -oestradiol is the initiator driver of CaP. In a study where CaP was induced in rodents, E2 carcinogenic effect was accelerated by estrogen¹².

However, one study questioned the opposite findings, which created low chances for the risk of prostate cancer and after deregulating E2 levels by upward adjustment of sex hormone-binding globulin¹³. It is argued that with the activities of SHBG and functionality of aromatase, the scientist should not point to testosterone elevation as a causative agent of prostate cancer, rather estrogen. This is because, at the steroidogenic pathway, elevated testosterone is aromatized to estrogen, profoundly by ER α as illustrated in Fig. 1.

This figure is a representation of the estrogen signaling mechanism in the prostate gland, where testosterone is aromatized to estrogen by the aromatase. This is done through estrogen receptor alpha (ER α) and beta (ER β). Cancer negative effect is observed at ER α in the stroma and epithelial cells including the aberrant proliferation of cell and inflammation. On the other hand, estrogen can exert beneficial or positive effects through ER β in the epithelia,

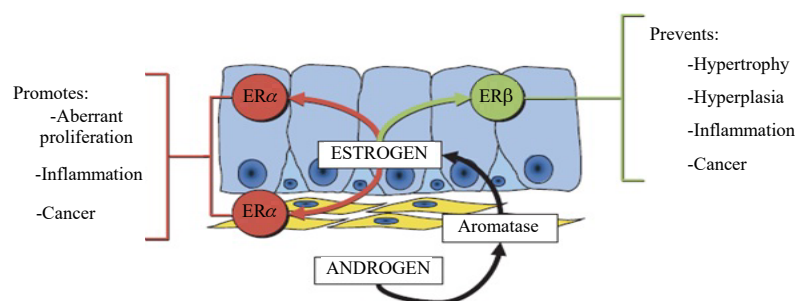


Fig. 1: Estrogen signaling mechanism

Source: Gann *et al.*¹³

which has been enlaced in the prevention of hyperplasia and hypertrophy, anti-proliferative and anti-carcinogens.

Besides, studies on rodent have proved a rising vulnerability to prostate cancer in mice that were exposed to a high dose of 17β -estradiol *in utero*¹⁴⁻¹⁵. The use of rodent-models may pave way to study the reproductive system after induction of estrogen. However, the prospect of making findings with specific species limits their utilization and correlation to humans. Testosterone is a precursor of estradiol- 17β in men. The activities of estrogens might be linked not only with the effect of ER α but with damaged DNA and functions estrogenic compounds¹⁶. There is a reason to believe that some negative effects of estrogen on the prostate gland are caused by exposures that may make one more likely to be affected by prostate disease later in life. One instance is the exposure-*in-utero* to diethylstilbestrol (DES) that was marked with an increase in breast and vaginal cancer of offspring. The exposure of adult-men to DES may have limited risk for cancers¹⁷. Depending on steroid hormones and imbalances of the prostate gland, abnormalities in cell growth and differentiation may still arise during development¹⁸⁻¹⁹. In another instance, the exposure-*in utero* to DES was marked with abnormalities at the prostatic utricle in newborn sons, but high maternal estrogen during pregnancy have shown increased prostate cancer risk in humans¹⁹. This implies that a total estrogen screening may be useful in identifying any type of uncontrolled tissue growth in the prostate before the proliferation manifest. The free Oestradiol in men's population of Eket-Nigeria was determined to establish the number of men with elevated Oestradiol that may be vulnerable to prostate proliferation.

MATERIALS AND METHODS

Research location: This research was done at Eket metropolis, in Akwa Ibom State, Nigeria. The investigation started on February 15 2016, after the collection of ethical approval.

Chemical grading: All chemicals used for this work are reagent of grade or higher, purchased from Reporter Assay Systems (AccuDiag™ ELISA).

Collection of blood serum: Blood samples from individuals of Eket were based on 197, using a simplified sample size formula, (Online sample Size Calculator by Raosoft, Inc.). The confidence level-n 95%, the margin of error-5%, population size-72, 856 and percentage population coverage 50%.

Collection of blood samples: The collection of blood samples was carried out by a Phlebotomist and prepared for hormonal assay.

Statistical analysis: Quantification of E2 and SHBG levels was carried out using ELISA. Concentrations of E2 and SHBG were interpolated by spline and lowess method with spline curve using graph prism version 6.5.

RESULT AND DISCUSSION

Figure 2 and 3 are the calibration curves of oestradiol and sex hormone binding globulin, where the absorbance-A 450 for each test and reference standard was plotted against concentration in pg mL^{-1} and mmol L^{-1} . The test and reference standards were interpolated.

According to the work published in Rev. Urology, the tissue or structural prevalence of BPH, which was catechized in autopsy, is comparatively 10% for men within the age-30s and 20% for men in their 40 years, but rises to 50-60% for men in their 60s and 80-90% for men in their 70 and 80s, respectively²⁰. When estrogen levels in men are higher than

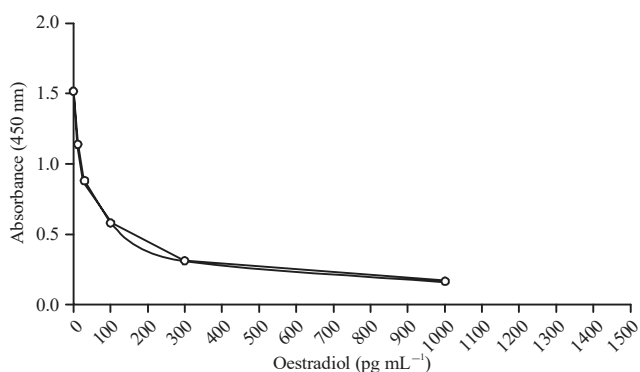


Fig. 2: Oestradiol calibration curve

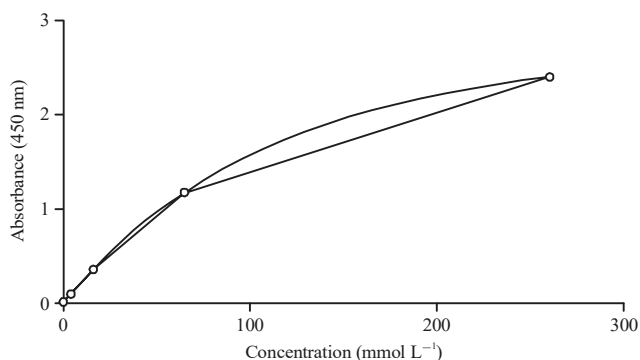


Fig. 3: SHBG calibration curve

they should, it can cause a slew of unwanted and dangerous side effects²¹. Some research has linked high estrogen to the enlargement of the prostate and prostate cancer. When the rise in estrogen is accompanied by a decline in testosterone, the risks are greater²².

Table 1 show total and free testosterone of 197 men of Eket-Nigeria. The explanation about why some men due suffer from surplus estradiol and fragmental testosterone is that during aging, their bodies generate lesser testosterone, while much of their helpful testosterone is proselytized to estradiol²³. Some researchers' asserted that elevated estrogen in men is the actual cause of prostate cancer due to

aromatization of testosterone to estrogen. The extreme indication of illness is the alteration of the ratio of testosterone to estrogen, which ultimately causes estrogen domination in men²⁴. This un-equilibrium between excess estrogen and paucity of testosterone is an overlooked causation of cardiac disease and infertility in men²⁵. Results of elevated estrogen levels as shown in Table 1 revealed that 28 men of 197 have elevated estrogen. This population accounted for 14.21% of 28/197 sampled population. Therefore, it can be inferred that men with elevated estrogen may possess the likelihood of ensuring prostate cancer and other diseases associated with estrogen or hormonal elevation. Long

Table 1: Oestradiol and SHBG concentration in men

Lab. No.	Age	E2 (pg mL ⁻¹)	SHBG (nmol L ⁻¹)	Free E2	Lab. No.	Age	E2 (pg mL ⁻¹)	SHBG (nmol L ⁻¹)	Free E2
1	48	5.0	27.5	5.50	44	56	8.3	17.3	2.08
2	30	9.2	15.0	1.63	45	67	2.3	27.8	12.08**
3	29	3.3	22.5	6.82	46	60	7.2	26.1	3.62
4	42	9.3	37.2	4.00	47	65	9.3	39.9	4.29
5	28	7.2	20.8	2.89	48	34	2.1	14.4	6.85
6	27	0.7	19.8	28.29**	49	33	2.3	12.6	5.47
7	25	2.1	36.9	17.57**	50	24	3.8	13.7	3.60
8	27	1.2	26.7	22.25**	51	31	9.2	15.9	1.72
9	35	5.6	27.9	4.98	52	30	2.3	8.7	3.00
10	28	3.1	25.6	8.26	53	45	3.2	19.3	6.03
11	34	7.5	40.2	5.36	54	55	2.9	8.9	3.07
12	82	1.8	12.9	7.17	55	47	4.7	22.0	4.68
13	30	2.5	15.2	6.08	56	49	8.4	11.1	2.30
14	27	2.2	10.6	4.82	57	50	6.7	14.1	2.98
15	29	3.9	34.3	8.79	58	50	8.2	22.1	1.32
16	34	1.6	9.1	5.68	59	54	5.9	39.7	6.72
17	45	1.2	5.3	4.41	60	44	2.7	27.7	10.25**
18	65	1.9	16.9	8.89	61	54	4.8	16.7	3.47
19	34	3.6	33.8	9.38	62	45	5.3	11.1	2.09
20	23	8.5	40.3	4.74	63	36	4.7	21.1	4.48
21	29	2.2	17.7	8.04	64	28	8.6	29.0	3.37
22	34	11.7	27.3	2.33	65	36	7.1	35.0	4.92
23	33	2.9	16.2	5.58	66	81	3.9	12.0	3.07
24	35	0.5	6.79	13.58**	67	56	5.0	12.0	2.40
25	25	17.6	19.1	1.08	68	35	6.9	16.8	2.43
26	46	25.7	40.4	1.57	69	38	2.0	12.0	6.00
27	43	4.1	29.8	7.26	70	29	3.6	15.0	4.16
28	22	0.2	1.3	6.50	71	34	1.9	12.9	6.78
29	21	8.2	17.0	2.07	72	54	3.8	18.8	4.94
30	37	6.1	12.9	2.11	73	65	5.4	12.89	2.38
31	56	2.8	2.9	1.03	74	72	0.9	4.8	5.33
32	65	6.2	24.8	4.00	75	23	4.0	22.88	5.72
33	45	0.9	3.7	4.11	76	38	7.3	26.0	3.56
34	33	2.1	8.9	4.23	77	29	2.8	12.0	4.28
35	44	0.6	1.9	3.17	78	30	8.9	54.0	6.06
36	56	0.8	1.89	2.36	79	37	2.8	11.9	4.25
37	23	2.8	3.78	1.35	80	36	5.0	15.0	3.00
38	44	9.4	19.9	2.11	81	29	6.6	13.1	2.62
39	22	3.2	20.3	6.34	82	30	4.6	24.0	7.86
40	34	1.8	11.1	6.17	83	40	4.4	9.78	6.06
41	34	1.9	22.3	11.73	84	44	3.3	14.0	9.42
42	43	3.3	22.9	6.94	85	21	6.9	13.9	4.92
43	65	3.7	4.0	1.08	86	36	2.8	27.0	4.57

Table 1: Continued

Lab. No.	Age	E2 (pg mL ⁻¹)	SHBG (nmol L ⁻¹)	Free E2	Lab. No.	Age	E2 (pg mL ⁻¹)	SHBG (nmol L ⁻¹)	Free E2
87	28	6.6	18.87	3.22	145	49	5.2	19.1	3.67
88	30	3.0	6.88	15.40**	146	50	3.5	40.4	11.54**
89	47	6.0	3.70	6.00	147	39	5.5	29.8	5.42
90	37	7.5	6.23	9.00	148	34	6.8	45.8	6.73
91	29	8.9	56.90	3.91	149	45	9.8	46.3	4.72
92	37	0.7	10.97	10.42**	150	55	2.1	37.9	18.04**
93	54	1.6	12.00	10.62**	151	64	8.2	67.9	8.28
94	56	0.5	2.60	11.40**	152	45	2.5	34.8	13.92**
95	22	4.3	38.90	5.88	153	72	1.0	7.3	7.30
96	37	4.0	17.90	4.72	154	56	1.3	17.0	13.08**
97	57	6.1	22.00	6.38	155	50	3.8	15.7	4.13
98	68	8.9	23.60	10.33**	156	46	4.8	25.3	5.27
99	55	5.2	15.34	1.96	157	49	1.05	18.9	18.00**
100	47	9.0	7.89	2.47	158	39	6.9	38.9	5.63
101	48	1.2	6.22	2.42	158	61	6.6	30.2	4.79
102	32	0.7	12.90	5.71	159	27	2.9	33.0	11.38**
103	26	2.4	17.30	7.20	159	48	2.6	11.5	4.42
104	37	6.4	2.30	4.34	160	40	5.8	43.2	7.44
105	45	3.4	35.30	7.67	160	28	4.4	43.0	9.77**
106	37	8.9	32.80	4.48	161	43	3.3	12.19	3.69
107	48	5.5	6.89	2.62	162	40	6.9	38.0	5.50
108	46	2.9	9.27	4.34	163	48	8.8	34.9	3.96
109	45	2.2	5.67	6.22	164	55	6.6	38.1	5.77
110	44	8.8	28.29	1.81	165	63	3.0	29.5	9.33**
111	53	3.2	17.29	2.72	166	23	6.0	25.6	4.20
112	51	6.2	22.90	3.11	167	39	7.5	51.3	6.84
113	38	4.9	17.89	1.81	168	44	8.9	30.6	3.43
114	46	5.5	43.90	4.00	169	42	7.5	53.0	7.06
115	33	5.2	26.30	2.13	170	34	1.6	13.6	8.50
116	34	6.2	24.70	2.27	171	24	5.5	23.8	4.32
117	39	3.9	21.30	0.54	172	30	4.3	15.6	3.62
118	25	3.6	2.11	1.02	173	38	4.0	16.4	4.10
119	22	2.4	2.11	1.12	174	44	6.1	35.7	5.85
120	38	8.8	3.79	1.89	175	37	8.9	67.90**	7.62
121	39	2.2	23.15	10.52**	176	30	5.2	15.9	3.05
122	31	4.9	19.00	3.88	177	36	9.0	79.8	8.86
123	31	5.9	42.50	7.20	178	45	1.2	11.9	9.92**
124	38	3.3	37.20	11.27**	179	34	1.7	12.5	7.35
125	40	8.2	20.80	2.53	180	53	4.4	39.9	9.06**
126	66	4.5	19.20	4.26	181	64	6.4	16.6	2.59
127	54	9.8	56.10	5.72	182	32	3.4	6.9	2.02
128	53	4.8	26.70	5.56	183	43	8.9	37.6	4.22
129	28	6.9	29.90	4.33	184	44	5.5	40.5	7.36
130	22	7.9	25.60	3.24	185	23	2.9	15.5	5.34
131	54	5.5	49.33	8.96	186	26	2.2	12.2	5.54
132	30	5.9	12.90	2.18	187	29	8.8	37.9	4.30
133	32	14.8	72.20	4.87	188	30	3.2	18.4	5.75
134	43	1.7	10.60	6.23	189	40	16.2	97.60**	6.02
135	28	2.7	34.30	12.70**	190	44	4.9	13.7	2.79
136	67	2.6	9.10	3.50	191	58	1.5	9.9	6.60
137	29	1.9	10.30	5.42	192	36	5.2	17.1	3.28
138	38	5.5	19.90	3.61	193	46	6.2	35.9	5.79
139	40	11.9	39.80	3.34	194	37	12.9	76.50**	5.93
140	41	7.4	46.70	6.31	195	52	3.7	65.9	17.81**
141	32	4.1	27.70	6.75	196	56	1.8	34.8	19.33**
142	57	1.2	27.30	22.75**	197	72	1.4	14.2	10.14**
143	60	3.8	11.20	2.95	Normal control		8.0	65.0	8.13
144	51	2.5	23.99	9.59	Abnormal control		154.6	67.1	0.43

E2: Estrogen concentration, SHBG: Sex hormone binding globulin, Free E2: Free estrogen concentration, **Values above normal range indicating estrogen elevation in men

before now, some investigators have queried the concept that testosterone is the first culprit in the causation of prostate cancer, that is because testosterone has been used as anti-estrogen therapy. They suggested that the increased risk of prostate cancer ranges from one's middle and senior years when testosterone is naturally declining and estradiol is gradually rising²⁶. On the abnormal side, the number of time or chances for prostate disease to occur usually increases with age. This is due to the decline in serum androgen levels. Consensus prove, suggests that estrogens may also be helpful in the normal health of the prostate, as well as in the etiology of prostate disease. Both estrogen receptor subdivision-ER α and ER β are present in prostate cancer development, which shows that the prostate gland responds in different ways to estrogens effect.

CONCLUSION

There are clearer prove that rising estradiol may be hard for the prostate gland and can pose a high risk to men. It is hoped that in-depth research will be carried out to ascertain just what a "high estradiol" level may cause in the prostate gland. Considerably, individual variability due occurs, while aromatase-enzyme that converts testosterone into estradiol is stored in fat cells. So the more body fat one has the more aromatase the person has and the higher the estradiol levels tend to be. Therefore, early detection or pre-cancers test can prevent the development of cancer, a form of primary prevention in this investigation.

SIGNIFICANCE STATEMENT

This study revealed that 28 adult men out of 197 have elevated estrogen beyond the recommended reference range 15-60 pg mL⁻¹, which implied that 14.21% of 28/197 men have elevated free estrogen. This study will help clinicians and researchers undertake routine screening on healthy and sick individuals to ascertain the safety level of their prostate. Also, it will be beneficial to the people of Eket to avoid diet and exposure to toxins that may cause estrogen elevation.

ACKNOWLEDGMENT

We sincerely appreciate the efforts of the Head of Department of Medical Laboratory Science and Hematology Unit, Mr. Akaka Christopher who help alongside his colleagues for collection of blood samples from donors and Miss Ada who help with serum extraction. We thank Dr. Emmanuel Nna of Safety Pathology Laboratory, who helps with analysis, order and safe handling of the ELISA kits.

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