Evaluation of some hormones in female having breast cancer before and after radiotherapy

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Abstract
Hormones are believed to largely affect cancer risk by controlling the rate of cell differentiation, division and quantity of cancer-vulnerable cells. Higher estrogen levels, progesterone, prolactin and serotonin have been linked to some cancers including breast cancer. The study aims for a relationship between estrogen, progesterone, prolactin and serotonin signaling and breast cancer to shed light on the pivotal role of hormonal imbalance in the development of breast cancer. The study included 50 new female patients with breast cancer, they do not have diseases related to hormones. Patients were followed up and blood samples were taken for the same group of patients when treated with radiation at the fifth and fifteenth doses (patients, R5, R15) with 50 samples of apparently healthy females as comparison group (control), The ages of the patients and healthy people ranged between 25-70 years. Estrogen, progesterone, prolactin and serotonin levels were tested and the results showed a significant increase in the concentration of estrogen at the probability level P<0.0001 for the three groups (patients, R5, R15) 596.8±107.6 pg/mL, 561.8±103.1 pg/mL, 466.9±80.43 pg/mL respectively compared to the control group of healthy women 178.3±69.74 pg/mL.

The results indicated a significant increase in the concentrations of progesterone, prolactin and serotonin at the probability level P<0.0001 for the three groups (patients, R5, R15) compared to the control group of healthy women and a significant decrease in the concentration of the hormone estrogen, progesterone, prolactin and serotonin at the probability level P<0.0001 for the group of women treated at the R-15 dose compared to the group of women treated at the dose R-5.

Keywords: Breast cancer, Estrogen, Progesterone, Prolactin, Serotonin.

Introduction

Breast cancer is one of the most commonly diagnosed cancers with a total of 2.3 million cases.[31] Breast cancer is a complicated disease with many risk factors and underlying mechanisms that contribute to its development and progression, one of these factors is hormonal imbalance which has a significant impact on the pathogenesis of breast cancer understanding the complex relationship between hormonal imbalance and the development of breast cancer is essential for furthering understanding of this disease and possibly developing better prevention and treatment strategies.[8,18,31] The aberrant proliferation of cells in the breast tissue is a hallmark of breast cancer.[2] It is the most prevalent type of cancer in women and the second largest cause of mortality.

Breast cancer is a disease that affects different populations around the world at different rates. Risk factors for the disease include age, family history and genetic mutations. The disease effects go beyond physical health, as it also affects psychological well-being and quality of life.[2,28] Many studies have looked into the relationship between progesterone and estrogen imbalance and breast cancer.[5] The hormone estrogen which is primarily produced in the ovaries is essential for the growth and development of breast tissue, likewise the menstrual cycle and the development of the breasts are regulated by progesterone another hormone that is mostly produced in the ovaries.[17] Breast cancer has been linked to disruptions in the delicate balance of these hormones, both in its initiation and progression.[13,34]

In order to successfully complete the cycles of mammary development and differentiation and to provide nourishment for the offspring prolactin collaborates with the ovarian steroids, estrogen and progesterone, PRL-initiated signals expand alveolar cells during pregnancy and coordinate their differentiation at the time of birth.[12,29] Epidemiological research demonstrates that prolactin plays a role in the development of breast tumors.[32,35] Crucial neurotransmitter serotonin is a hormone that acts independently of the central nervous system.[10] Long recognized to be crucial for platelet aggregation and vasoconstriction, serotonin has also been demonstrated to have additional functions in metabolism.[15] Thus, serotonin not only stimulates the growth of tumor cells but also controls tumor growth by controlling angiogenesis, invasion, metastasis and cell proliferation.[26,37]

Material and Methods

Collection of Samples: Blood samples of breast cancer female patients (25-70 years) were obtained from the Radiation Center for the treatment of cancerous tumors - Sulaymaniyah and Teaching Oncology Hospital at the City of Medicine and the National Center for Oncology - Baghdad, After ethical authorization the patients, blood samples were taken from women after diagnosis of the disease (new case) before radiation treatment and then

females with breast cancer were followed up for the same cases after radiation treatment. Blood samples were taken from female with breast cancer for two doses of radiation treatment and the treatment protocol was chosen.

In fifteen doses for 3 weeks, it was in the form of daily doses using a CT-Simulation device and the amount of dose was 40Gy/15fx, which corresponds to 2.67Gy/day, as blood samples were taken for patients treated with radiation in the fifth dose and the fifteenth dose. The total of the samples was 150 samples. We also collected 50 blood samples from apparently healthy females who do not have cancerous or other inflammatory diseases and do not have a family history as a control group.

Measuring the level of hormones in the blood serum of the samples under study: Hormones are estimated in the blood serum of the groups under study by following the steps attached to the special examination kit according to the ELASIA technology, which is based on the principle of the double antibody sandwich technique, by means of immunoabsorption. Samples containing antigens and antibodies bound to biotin are added to the antibodies. In the Elisa plate wells, the plates are washed with buffer washing solution to remove unbound antibodies, then the conjugated enzyme Avidin-peroxidase conjugates are added and the TMB base material is used for staining. Thus, TMB reacts with the peroxidase enzyme, this leads to the appearance of a blue color and it eventually turns yellow after adding the stopping solution.[14,30]

Results and Discussion

Estimating the concentration of Estrogen hormone in blood serum: The mean±SD of estrogen concentrations in healthy female (control) and barest cancer female patients, R5 and R15 groups were 178.3±69.74 pg/mL, 596.8±107.6 pg/mL, 561.8±103.1pg/mL, 466.9±80.43 pg/mL respectively as in figure 1. The results showed that estrogen levels increased significantly ($p<0.0001$) in patients compared to the control group and results showed that estrogen levels decreased significantly ($p<0.0001$) in R15 group compared to the patient group.

The results of the current study are consistent with the results of El-Aaser et al.[7] In a study they researched on women with breast cancer having age between 20-29 years during menstruation, the total estrogen level showed a significant increase in the early follicular phase compared to healthy women. In women with breast cancer after menopause, the total estrogen level showed a significant increase compared to the hormone level in normal healthy women.

Quigley et al[25] also reported in a histological study of breast cancer patients that there was an inverse relationship between the increase in tumor estrogen pathway expression and the degree of tumor macrophages compared with adjacent normal tissue.

Estimating the concentration of Progesterone hormone in blood serum: The mean±SD of progesterone concentration in healthy female (control) and breast cancer female patients, R5 and R15 groups were 4.212 ±2.218 ng/mL, 43.83±10.09 ng/mL, 41.47±8.227 ng/mL, 31.58 ±6.029 ng/mL respectively as in the figure 2. The results showed that progesterone levels increased significantly ($p<0.0001$) in patients compared to the control group and results showed that progesterone levels decreased significantly ($p<0.0001$) in R15 group compared to the patient group.

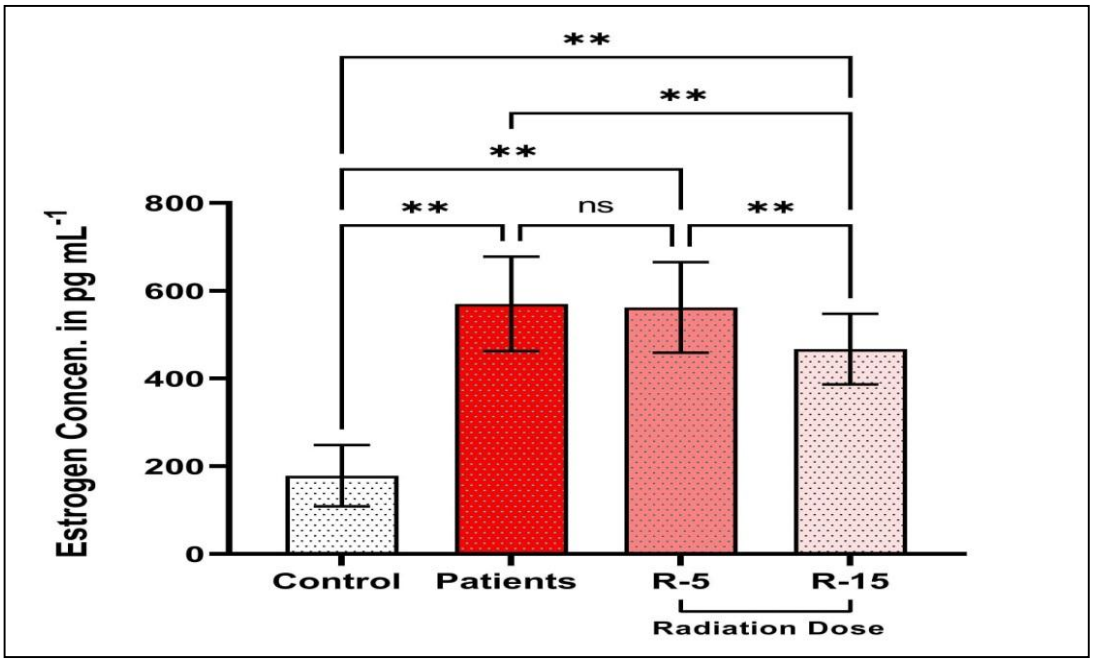

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Figure 1: Levels of estrogen for control, patients, R5 and R15 under study groups

The results of the current study are consistent with those of Trabert and his group.[33] In a study they examined 405 cases of breast cancer. The cases were followed up over a period of 12 years and another group that included 445 postmenopausal women who did not use exogenous hormone therapy, in which they concluded that high progesterone levels are associated with an increased risk of breast cancer by 16%.

In the same study, progesterone and estradiol were examined together and it was found that higher concentrations of progesterone led to an increased risk of breast cancer with an increase in estradiol concentration.[33] In recent years, studies have shown that progesterone expands the pool of stem cells sensitive to transformation. This has led to hypotheses that progesterone may prepare the breast for carcinogenesis by activating the pool of stem cells and accelerating tumor formation.[4,16]

Estimating the concentration of Prolactin hormone in blood serum: The mean±SD of prolactin concentrations in healthy female (control) and breast cancer female patients, R5 and R15 groups were 18.24 ±4.900 ng/mL,182.1 ±12.21 ng/mL, 182.0±11.23 ng/mL, 173.3±24.6 ng/mL respectively as in figure 3. The results showed that prolactin levels increased significantly ($p<0.0001$) in patients compared to the control group and results showed that prolactin levels decreased significantly ($p<0.0001$) in R15 group compared to the patient group.

The results of the current study are consistent with a study in the city of Karbala on 71 women with breast cancer who were diagnosed in Al-Hussein Hospital. There was a relationship of high moral value between the level of the hormone prolactin and breast cancer.[1] Aranha et al[3] pointed out in a statistical study called meta-analysis for the purpose of integrating the qualitative and quantitative data of these studies with overlapping results and reaching accurate statistical conclusions. They found through this study a positive relationship between levels of the hormone prolactin in plasma and breast cancer, especially in invasive breast cancer in postmenopausal age.

The results of the current study are also consistent with what Mulani et al[19] found in a study conducted in the city of Nagpur in India on breast cancer patients, patients suffering from benign breast diseases, as a control group. The results of the study summarized that cancer patients breasts have increased levels of the hormone prolactin in the blood compared to patients with benign breast diseases.

Estimating the concentration of Serotonin hormone in blood serum: The mean±SD of serotonin concentration in healthy female (control) and breast cancer female patients, R5 and R15 groups were 86.75±26.97 ng/dmL, 270.8±43.05 ng/mL, 247.7±53.26ng/mL, 190.5±26.89 ng/mL respectively as in the figure 4. The results showed that serotonin levels increased significantly ($p<0.0001$) in patients compared to the control group and results showed that serotonin levels decreased significantly ($p<0.0001$) in R15 group compared to the patient group.

The results of the current study are consistent with a study that identified serotonin as a predictive marker for breast cancer patients using different types of breast cancer cell lines where serotonin expression was increased in human breast cancer cell lines compared to human breast epithelial cell lines.[36]

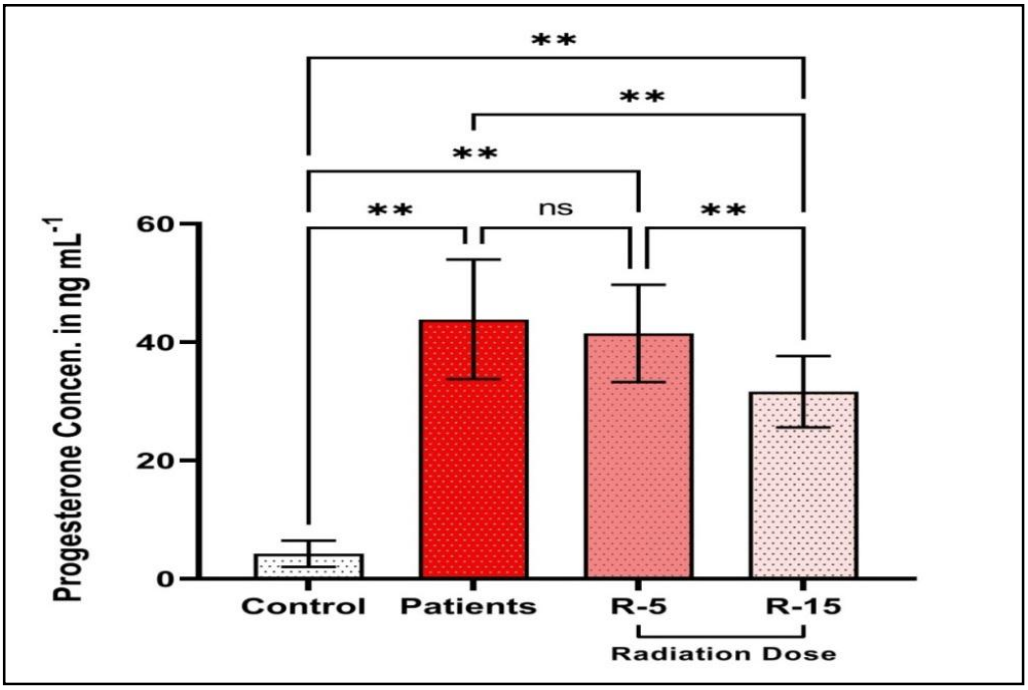

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Figure 2: Levels of progesterone for control, patients, R5 and R15 under study groups

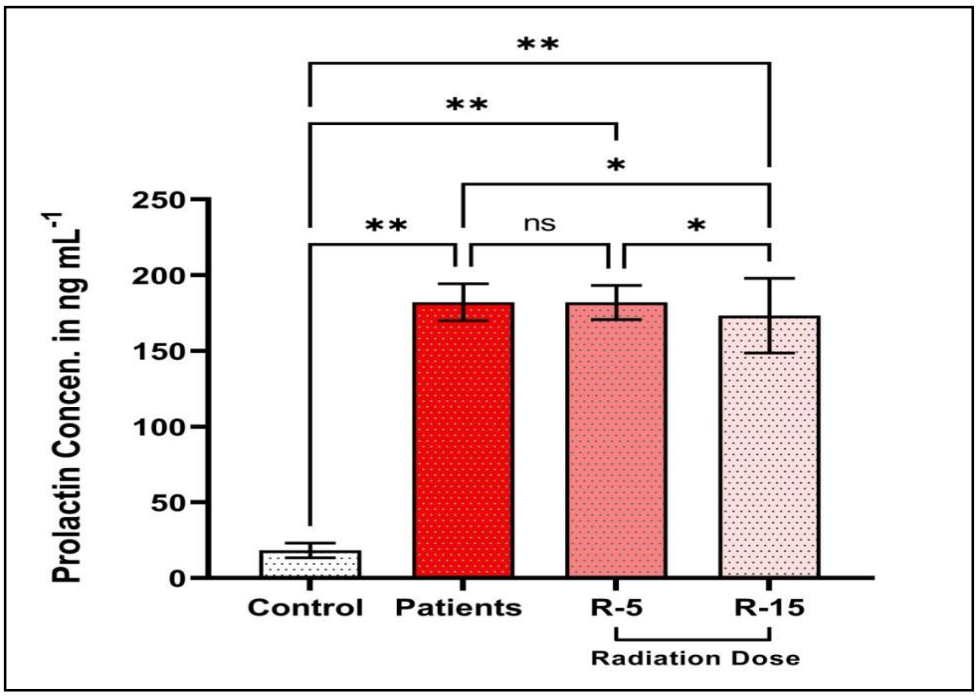

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Figure 3: Levels of prolactin for control, patients, R5 and R15 under study groups

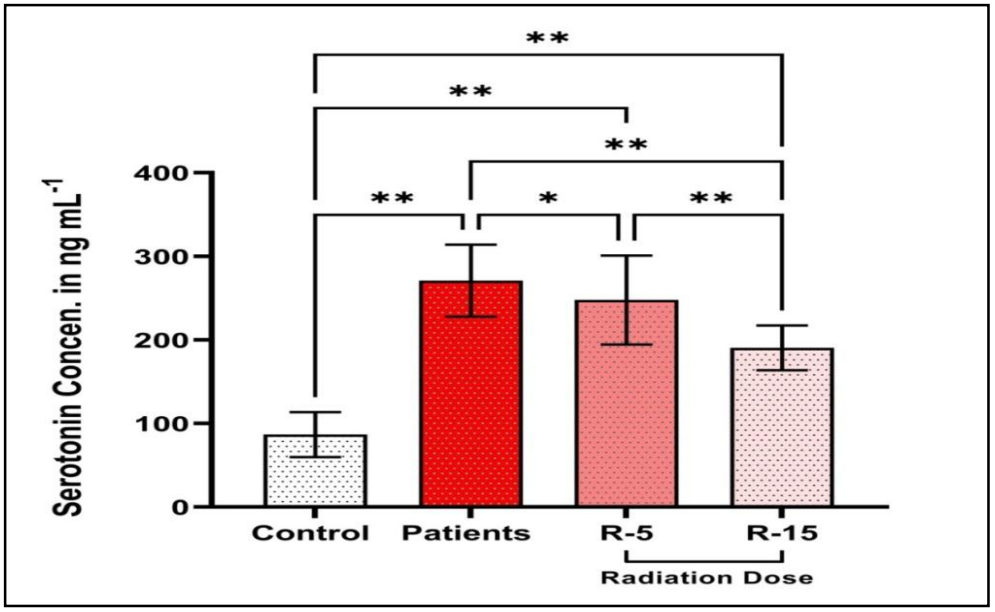

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Figure 4: Levels of serotonin for control, patients, R5 and R15 under study groups

The results of recent studies largely emphasized the important role of serotonin in regulating normal mammary gland tissue and breast cancer.[27] Preclinical studies have discovered that serotonin affects tumor angiogenesis and tumor growth.[35,36] In addition, angiogenesis is one of the hallmarks of cancer and is a pre-requisite for tumor growth because it provides oxygen and nutrients and removes decomposition products from the tumor microenvironment.[11]

References

1. Ali J.K., Hassan S.H. and Merzah M.A., Prolactin serum levels and breast cancer: Relationships with hematological factors among cases in Karbala Province, Iraq, *Medical Journal of Babylon*, **15(2)**, 178-18 **(2018)**

2. Alkabban F.M. and Ferguson T., Breast Cancer, 2022 Sep 26, In StatPearls [Internet], Treasure Island (FL): StatPearls Publishing **(2023)**

3. Aranha A.F., Dos Anjos L.G., Turri J.A.O., Simões R.S., Maciel G.A.R., Baracat E.C., Soares-Júnior J.M. and Carvalho K.C., Impact of the prolactin levels in breast cancer: a systematic review and meta-analysis, *Gynecological Endocrinology: The Official Journal of the International Society of Gynecological Endocrinology*, **38(5)**, 385-390 **(2022)**

4. Asselin-Labat M.L., Vaillant F., Sheridan J.M., Pal B., Wu D., Simpson E.R., Yasuda H., Smyth G.K., Martin T.J., Lindeman G.J. and Visvader J.E., Control of mammary stem cell function by steroid hormone signaling, *Nature*, **465(7299)**, 798-802 **(2010)**

5. Brisken C. and O'Malley B., Hormone action in the mammary gland, *Cold Spring Harbor Perspectives Biology*, **2(12)**, a003178 **(2010)**

6. Check J.H., Ubelacker L. and Lauer C.C., Falsely elevated steroidal assay levels related to heterophile antibodies against various animal species, *Gynecologic and Obstetric Investigation*, **40(2)**, 139-40 **(1995)**

7. el-Aaser A.A., el-Merzabani M.M. and Abu-Bedair F.A., Serum estrogen level in Egyptian breast cancer patients, *Tumori*, **71(3)**, 293-5 **(1985)**

8. Feng Y., Spezia M., Huang S., Yuan C., Zeng Z., Zhang L., Ji X., Liu W., Huang B., Luo W., Liu B., Lei Y., Du S., Vuppalapati A., Luu H.H., Haydon R.C., He T.C. and Ren G., Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics and molecular pathogenesis, *Genes & Diseases Journal*, **5(2)**, 77-106 **(2018)**

9. Graham J.D., Mote P.A., Salagame U., van Dijk J.H., Balleine R.L., Huschtscha L.I., Reddel R.R. and Clarke C.L., DNA replication licensing and progenitor numbers are increased by progesterone in normal human breast, *Endocrinology*, **150(7)**, 3318-26 **(2009)**

10. Gwynne W.D., Shakeel M.S., Girgis-Gabardo A. and Hassell J.A., The Role of Serotonin in Breast Cancer Stem Cells, *Molecules (Basel, Switzerland)*, **26(11)**, 3171 **(2021)**

11. Hanahan D. and Weinberg R.A., Hallmarks of cancer: the next generation, *Cell*, **144(5)**, 646-74 **(2011)**

12. Horseman N.D., Prolactin and mammary gland development, *Journal of Mammary Gland Biology and Neoplasia*, **4(1)**, 79-88 **(1999)**

13. Jamie D., Invasive Ductal Carcinoma (IDC): Grade, Symptoms & Diagnosis **(2023)**

14. Jeong J.J., Ganesan R., Jin Y.J., Park H.J., Min B.H., Jeong M.K., Yoon S.J., Choi M.R., Choi J., Moon J.H., Min U., Lim J.H., Lee D.Y., Han S.H., Ham Y.L., Kim B.Y. and Suk K.T., Multi-strain probiotics alleviate loperamide-induced constipation by adjusting the microbiome, serotonin and short-chain fatty acids in rats, *Frontiers in Microbiology*, **14**, 1174968 **(2023)**

15. Jones L.A., Sun E.W., Martin A.M. and Keating D.J., The ever-changing roles of serotonin, *The International Journal of Biochemistry & Cell Biology*, **125**, 105776 **(2020)**

16. Joshi P.A. et al, Progesterone induces adult mammary stem cell expansion, *Nature*, **465(7299)**, 803-7 **(2010)**

17. Lange C.A. and Yee D., Progesterone and breast cancer, *Women's Health (London England)*, **4(2)**, 151-62 **(2008)**

18. Łukasiewicz S., Czeczelewski M., Forma A., Baj J., Sitarz R. and Stanisławek A., Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers and Current Treatment Strategies-An Updated Review, *Cancers*, **13(17)**, 4287 **(2021)**

19. Mulani J.D., Murhar B., Jambhulkar R. and Mishra G., Serum Prolactin: A Clue to Breast Malignancy, *Medical Laboratory Journal*, **16(1)**, 13-19 **(2022)**

20. Oakes S.R., Rogers R.L., Naylor M.J. and Ormandy C.J., Prolactin regulation of mammary gland development, *Journal of Mammary Gland biology and Neoplasia*, **13(1)**, 13-28 **(2008)**

21. Obr A.E., Grimm S.L., Bishop K.A., Pike J.W., Lydon J.P. and Edwards D.P., Progesterone receptor and Stat5 signaling cross talk through RANKL in mammary epithelial cells, *Molecular Endocrinology Journal*, **27(11)**, 1808-24 **(2013)**

22. Pasupuleti Vidya Sagar Venkata, Bagade Prashant and Kachireddy Naga Suresh Reddy Venkata, A Sensitive and High Throughput LC-MS/MS Method for the Determination of Resveratrol in Peanut Matrix, *Res. J. Chem. Environ.*, **27(9)**, 46-52 **(2023)**

23. Pompili M., Serafini G., Palermo M., Seretti M.E., Stefani H., Angeletti G., Lester D., Amore M. and Girardi P., Hypothalamic pituitary adrenal axis and prolactin abnormalities in suicidal behavior, *CNS & Neurological Disorders Drug Targets*, **12(7)**, 954-70 **(2013)**

24. Qin L., Zhao D., Xu J., Ren X., Terwilliger E.F., Parangi S., Lawler J., Dvorak H.F. and Zeng H., The vascular permeabilizing factors histamine and serotonin induce angiogenesis through TR3/Nur77 and subsequently truncate it through thrombospondin-1, *Blood*, **121(11)**, 2154-64 **(2013)**

25. Quigley D.A., Tahiri A., Lüders T., Riis M.H., Balmain A., Børresen-Dale A.L., Bukholm I. and Kristensen V., Age, estrogen and immune response in breast adenocarcinoma and adjacent normal tissue, *Oncoimmunology*, **6(11)**, e1356142 **(2017)**

26. Sarrouilhe D., Clarhaut J., Defamie N. and Mesnil M., Serotonin and cancer: what is the link?, *Current Molecular Medicine*, **15(1)**, 62-77 **(2015)**

27. Sarrouilhe D. and Mesnil M., Serotonin and human cancer: A critical view, *Biochimie*, **161**, 46-50 **(2019)**

28. Satpathi S., Gaurkar S.S., Potdukhe A. and Wanjari M.B., Unveiling the Role of Hormonal Imbalance in Breast Cancer Development: A Comprehensive Review, *Cureus Journal*, **15(7)**, e41737 **(2023)**

29. Shin H.Y., Hennighausen L. and Yoo K.H., STAT5-Driven Enhancers Tightly Control Temporal Expression of Mammary-Specific Genes, *Journal of Mammary Gland Biology and Neoplasia*, **24(1)**, 61-71 **(2019)**

30. Soltysik K. and Czekaj P., Membrane estrogen receptors - is it an alternative way of estrogen action?, *Journal of Physiology and Pharmacology: An Official Journal of the Polish Physiological Society*, **64(2)**, 129-42 **(2013)**

31. Sung H., Ferlay J., Siegel R.L., Laversanne M., Soerjomataram I., Jemal A. and Bray F., Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for

36 Cancers in 185 Countries, *CA: A Cancer Journal for Clinicians*, **71(3)**, 209-249 **(2021)**

32. Tikk K., Sookthai D., Johnson T., Rinaldi S., Romieu I., Tjønneland A., Olsen A., Overvad K., Clavel-Chapelon F., Baglietto L., Boeing H., Trichopoulou A., Lagiou P., Trichopoulos D., Palli D., Pala V., Tumino R., Rosso S., Panico S., Agudo A., Menéndez V., Sánchez M.J., Amiano P., Huerta Castaño J.M., Ardanaz E., Bueno-de-Mesquita H.B., Monninkhof E., Onland-Moret C., Andersson A., Sund M., Weiderpass E., Khaw K.T., Key T.J., Travis R.C., Gunter M.J., Riboli E., Dossus L. and Kaaks R., Circulating prolactin and breast cancer risk among pre- and postmenopausal women in the EPIC cohort, *Annals of Oncology: Official Journal of the European Society for Medical Oncology*, **25(7)**, 1422-1428 **(2014)**

33. Trabert B., Bauer D.C., Buist D.S.M., Cauley J.A., Falk R.T., Geczik A.M., Gierach G.L., Hada M., Hue T.F., Lacey J.V., LaCroix A.Z., Tice J.A., Xu X., Dallal C.M. and Brinton L.A., Association of Circulating Progesterone with Breast Cancer Risk Among Postmenopausal Women, *JAMA Network Open*, **3(4)**, e203645 **(2020)**

34. Trabert B., Sherman M.E., Kannan N. and Stanczyk F.Z., Progesterone and Breast Cancer, *Endocrine Reviews*, **41(2)**, 320-44 **(2020)**

35. Tworoger S.S., Eliassen A.H., Zhang X., Qian J., Sluss P.M., Rosner B.A. and Hankinson S.E., A 20-year prospective study of plasma prolactin as a risk marker of breast cancer development, *Cancer Research*, **73(15)**, 4810-9 **(2013)**

36. Xie Q.E., Du X., Wang M., Xie F., Zhang Z., Cao Y. and Liu Y., Identification of Serotonin as a Predictive Marker for Breast Cancer Patients, *International Journal of General Medicine*, **14**, 1939-1948 **(2021)**

37. Ye D., Xu H., Tang Q., Xia H., Zhang C. and Bi F., The role of 5-HT metabolism in cancer, *Biochimica et biophysica acta, Reviews on Cancer*, **1876(2)**,188618 **(2021)**.

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