Genetic study of different genes in the formation and metabolism of neurosteroids and cholesterol.

Jenan M. Mohsen Al Mosawi[1] , Alaa Abdul Razzaq Al Nuaimi[2], Farah Riadh Zayny[3]

1Department of Pharmaceutics, Collage of Pharmacy, University of Karbala, Iraq, 2Ministry of health, Karbala health directorate, Imam Al-Hussein Medical-City, iraq, 3 Department of Pharmaceutical Biosciences , Faculty of Pharmacy, Uppsala university, sweden.

Abstract :

Some of the neurodegenerative diseases is Parkinson's disease and Alzheimer's disease, which can arise due to damage to nerve cells in the elderly. This study examines the different genes that express enzymes for the formation and metabolism of neurosteroids and cholesterol. The purpose of the study is to investigate which genes are expressed in the CNS, and that could affect the functions of the brain via the formation and metabolism of neurosteroids. The study used the human cell line SH-SY5 as the model for neuronal cells. By knowing which genes nerve cells express would in future be able to study the effect of different drugs can have in the formation of steroid metabolites that affect brain functions.

Methods used in this study, RT-PCR (reverse transcription polymerase chain reaction) and gel electrophoresis to qualitatively examine gene expression in neuroblastoma. Results from the study suggested that SH-SY5Y cells express some but not all genes involved in the formation and metabolism of steroid hormones and cholesterol. PCR experiments, showed expression of the genes for the enzymes CYP19A1 and 17-hydroxysteroid dehydrogenase (17β-HSD) and the estrogen receptor ER-β.

One conclusion that can be drawn from the results of this study is to neuroblastoma cell line SH-SY5Y may constitute a possible cell model for future research on hormonal effects and the effect of various drugs on the formation of neurosteroids that influence brain functions.

The purpose of this study is to investigate whether the SH-SY5Y cells (neuroblastoma cells) can express genes required for the formation and metabolism of cholesterol and steroid hormones (neurosteroids). The study also intends to investigate which genes can participate and which is expressed in the formation of steroid metabolites that affect brain functions.

دراسة وراثية عن الجينات المختلفة في تشكيل والتمثيل الغذائي للستيرويدات العصبية والكوليسترول.

جنان محمد محسن آلموسوي[1] علاء عبد الرزاق محمد النعيمي[2] ، فرح رياض زيني[3].

1 فرع الصيدلانيات، كلية الصيدلة، جامعة كربلاء، العراق، 2 وزارة الصحة ،دائرة صحة كربلاء ، مدينة الامام الحسين الطبية ، العراق , 3 قسم العلوم البيولوجية ، كلية الصيدلة، جامعة أوبسالا، السويد.

الكلمات المفتاحية :الستيرويدات العصبية ، الجين ، تفاعل البلمرة المتسلسل ، الكوليسترول,ايستروجين.

الخلاصة:

تعد بعض أمراض الاعصاب مثل مرض باركنسون ومرض الزهايمر، والتي يمكن أن تنشأ نتيجة لتلف خلايا العصبية في كبار السن ، حيث تبحث هذه الدراسة الجينات المختلفة التي تعبر عن الانزيمات للتشكيل والتمثيل الغذائي للستيرويدات العصبية والكوليسترول. والغرض من هذه الدراسة هو التحقيق في الجينات التي يتم التعبير عنها في الجهاز العصبي المركزي، والتي يمكن أن تؤثر على وظائف الدماغ عن طريق تشكيل والتمثيل الغذائي للستيرويدات العصبية .واستخدمت الدراسة خط الخلية البشرية SH-SY5 كنموذج للخلايا العصبية. من خلال معرفة الجينات التي تعبر الخلايا العصبية سيكون في المستقبل تكون قادرة على دراسة تأثير الأدوية المختلفة يمكن أن يكون في تكوين مستقبلات الستيرويد التي تؤثر على وظائف الدماغ.

الأساليب المستخدمة في هذه الدراسة، استخدام الناسخ العكسي لتفاعل البلمرة المتسلسل (RT-PCR) والجل الكتروفوريسيز لدراسة التعبير الجيني نوعيا للجينات العصبية.

ان نتائج الدراسة أشارت إلى أن الخلايا SH-SY5Y تعبر عن بعض وليس كل الجينات المسؤولة عن تكوين والتمثيل الغذائي للهرمونات الستيرويد والكوليسترول.ان تجارب ال PCR ، والتي بينت التعبير عن الجينات للانزيمات CYP19A1 و (17β-HSD) 17-hydroxysteroid dehydrogenase ومستقبلات هرمون الاستروجين.ER-β.

ان أحد الاستنتاجات التي يمكن استخلاصها من نتائج هذه الدراسة هو خط الخلية العصبية SH-SY5Y التي من الممكن ان تشكل نموذجا كخلية للبحوث المستقبلية على التأثيرات الهرمونية وتأثير الادوية المختلفة على تشكيل للستيرويدات العصبية التي تؤثر على وظائف المخ.

والغرض من هذه الدراسة هو التحقيق في ما إذا كانت الخلايا SH-SY5Y (الخلايا العصبية) يمكن التعبير عن الجينات اللازمة لتشكيل واستقلاب الكوليسترول وهرمونات الستيرويد (neurosteroids) .

كما تهتم هذه الدراسة أيضا إلى التحقيق عن الجينات التي يمكن أن تشارك والتي يعبر عنها في تكوين مستقبلات الستيرويد التي تؤثر على وظائف الدماغ.

Introduction:

Steroid hormones and neuro-degenerative diseases.

Steroid hormones are formed from cholesterol and the like synthesized by enzymes in the CNS called neurosteroids. These enzymes catalyze reactions in nerve cells and in other cell types to form steroid hormones such as progesterone, estradiol and

testosterone. Neurosteroid is that they modulate neurotransmission and myelination in the CNS. Changes in steroidmetaboliserande enzymes affecting levels of

neurosteroids which in turn leads to the brain function can be affected by neurodegenerative disorders. There are changes in neurosteroid modulating the GABA-A receptor. Examples of neurodegenerative diseases is Parkinson's disease, multiple sclerosis (MS) and Alzheimer's disease. They do not yet know everything about which of the genes and enzymes needed for the formation of steroid hormones, which are expressed in the brain's nerve cells [23].

Alzheimer's disease (AD)

AD is one of the degenerative diseases can not be cured. Deposition of β-amyloid (Aβ) takes place during the first stage. In this stage, the patient no dementia, but only a problem with the cache i.e. remembering things. In the second stage proceeds disease process [1].

Reasons for this is that the nerve cells in the CNS damaged when the protein β-amyloid accumulates in senile plaques. β-amyloid is a part of the amyloid precursor protein (APP) which is a transmembrane protein. Aβ is cleaved by various enzymes α-secretase, β-secretase and γ-secretase. α-secretase cleaves APP in the middle leading to Aβ plaques do not accumulate in healthy subjects. The frequency of this splitting increases as you get older, the large amounts of amyloidaplack accumulate in the brain and the production of α-secretase decreases, while there is an increase in β-secretase and γ-secretase. Other reasons for the patient develops AD can be increased oxidative stress in the brain and the ability to stimulate the formation of free radicals, reduced levels of polyunsaturated fatty acids in the brain, increased lipid peroxidation with increased level of 4-hydroxynonenal i.e. an aldehyde of lipid peroxidation in the ventricular fluid, reduced energy metabolism, decreased concentration of cytochrome c oxidase in the brain. Studies have shown that amyloid may generate free radicals. This oxidative stress can cause the cells to undergo apoptosis, which may occur in neurodegenerative diseases such as e.g. Alzheimer's disease and Parkinson's disease.

Tau protein is a normal occurring phosphorus protein that is synthesized in neurons. The protein may increase the stability of the neuron by binding to microtubules in axons, which form part of the cytoskeleton. It transports nutrients from neuron nucleus to the synapse.

Parkinson's disease:

Parkinson's disease may result from pharmacological treatment, cholinergic deficiency, reduced amount of dopamine (D2) - receptors and the presence of alpha-synuclein in Lewy bodies. Alfasynuklein is a protein in neurons affecting mitochondria. This can lead to inhibition of mitochodrial complex in the electron transport, which in turn leads to reactive oxygen species formed when the cell is exposed to oxidative stress, membranpermiabilitet and release of cytochrome c increased which leads to degraded neurons [4].

Some Genes involved with disease and examined in the study:

1) 17β-hydroxysteroid dehydrogenase (17β-HSD): 17β-hydroxysteroid dehydrogenases, also called 17-keto steroid reductases (17-KSR), the enzyme that performs conversion of androstenediol, estrone, DHEA,

androstenedione, testosterone and estradiol, such as the conversion of androstenedione to testosterone. The enzyme catalyzes the dehydrogenation of 17-hydroxy steroids in steroidogenesis [20]. There are 7 different types of 17β-hydroxysteroid dehydrogenases (type 1-7) [23].

2) Aromatase (CYP19A1)

It is a cytochrome P450 enzyme belonging to the family 19A. The protein is known that estrogen synthase, can synthesize estrogen, estradiol from androgens. Aromatase is an enzyme which catalyzes the biosynthesis of estradiol and produced by the CYP19A1 gene. Both the amygdala and prefrontal cortex neurons, astrocytes express aromatase. Estradiol is part of the hormonal system that has a major role in the neuronal survival and neuroprotective effect [6].

3) CYP17A1

CYP17A1 is an enzyme that converts forming precursors which are important in the formation of estrogen and testosterone. The enzyme catalyzes the reaction which 17OH-pregnenolone is converted into DHEA. Progesterone and pregnenlon converted to 17OH progesterone and 17OH-pregnenolone [7].

4) ER-α and ER-β

A biological effect occurs when estrogen binds to its specific estrogen receptors (ER). The receptors are expressed in two different genes, ER-α and ER-β, which means they are available in two different subtypes. These receptors act as ligand-regulating transcription factors and belong to the nuclear receptors and because they are expressed in various tissues may be different biological effects [9]. Estrogens regulate the growth and differentiation of dendrites and axons in the brain [8].

5) 3β-HSD (type 1 and 2)

3β-hydroxysteroid dehydrogenase (3β-HSD) occurs in two isoenzyme forms, namely 3β-HSD type 1 and 3β-HSD type 2. Type 1 is expressed in placenta, type 2 is expressed in the adrenal gland, testes and ovary. [10].

6) CYP11A1

CYP11A1 is an enzyme which carries out the first reaction in the metabolism of cholesterol to steroid hormones. The enzyme converts cholesterol to pregnenolone is a precursor to all the other corticoids [11].

Materials and Methods

RT-PCR:

RNA (1μg) extracted from different human tissue samples (biopsy of 3 man and 3 woman) such as nerve cells (SH-SY5Y) , and then used ME1 program (PrimePCR ™ preamp SYBR® Green Assay: ME1, Human ,70C^o for 10 min) in the PCR machine to make cDNA. RNA was then centrifuged where upon it was placed in ice. A reaction mix consisting of 1μg RNA was denatured by heating at 70C^o for 10 minutes, 2μl of dNTP mix, 4 μl MgCl 2, 2 μl10x RT buffer, 1 μl oligo (dT) primers, nuclease-free water to a final volume of 20 V, 0.5 μl RNAsin Ribonuclease inhibitor and 1 μl Revese transcriptase. To tllverka cDNA, ME2 program (room temperature 10 minutes, 420 for 15 min, 950 for 5 min, 0-50 in 5min). Where of the PCR program. A PCR reaction was mixed. This is composed of specific primer (table 1), 5 μl template cDNA from the RT reaction, 5 μl MgCl 2, 1 μl dTNP mix (10 mM), 5 μl 10xPCR bf 2 μlFWD Primer (10-50 pmol), 2 μlPrimer REV (10-50 pmol), nukleasfrittt water to a final volume of 50 μl. To this was added 0.5 μl of Taq DNA polymerase.

PCR cycles were used with different programs depending on which genes express some primers as in table1 and table 2.

Finally gel electrophoresis was performed on the PCR products, using the UV transilluminator visualized RNA bands from different tissues (human liver cells, DU145, ovaries, placenta, testicles and human neurons SH-SY5Y). As positive controls in PCR experiments, RNA from various tissues that are human liver cells, DU 145 prostate cells, ovary, placenta, testes. The negative control was untreated ie it had the same blend as other PCR reactions without the cDNA, ie it consisted only of the PCR reaction. Instead of cDNA, 5 μl of water. A negative control are executed in order to ensure the result obtained by other primers (tab2).

Table 1. Different PCR program for various human primers

Gener	Primer	PCR- program	Reference
17β-HSD **typ 5**	(F) CCAGGTGAGGAACTTTCACCAA (R) TGGCCAATCCTGCATCCTT	95C^0 10 min, 94C^0 1min, 55C^0 1 min, 72C^0 1min, 35 times to 2 ,72C^0 10min, 4C^0	13
CYP19A1	(F)TGTCTCTTTGTTCTTCATGCTATTTCTC (R) TCACCAATAACAGTCTGGATTTCC	95C^0 10 min, 94C^0 1min, 60C^0 1 min, 72C^0 1min, 45 times to 2,72C^0 10min, 4C^0	14

CYP17A1	(F) GCG ATC AGA AGC TGG AGA AG (R) CCA CAG AGG TGG TGG TCT C	95C° 10 min, 94C° 1 min, <u>59C° 1 min</u>, 72C° 1 min, <u>35 times to 2</u>, 4C°	15
CYP17A1	(F) GCCTCCTTGTGCCTAGAGTT (R) AAATAAGCTAGGGTAAGCAGCAAG	50C° 2 min, 95C^0 10min, 95C^0 15 Sec, 66C° 1 min, 72C° 1 min, <u>45 times to 3</u>, 4C°	16
ER-α	(F) ACAAGGGAAGTATGGCTATGCGCAAGTGAAATC TCCTCCG (R) CATCTCTCTGGCGCTTGTGTTCTGTCAATGCAGTT TGTAG	94° C for 45 s , 55°C 45 s, 28 or 38 cycles and 72°C 90 s	9
ER-α	(F) CCACCAACCAGTGCACCATT (R) GGTCTTTCGTATCCCACCTTTC	94° C for 15 , 94°C 15s, 56 ^{0} C 20 sec, 50 times to 2, 72°C 10 sec, 4	18
ER-β	(F) TAGTGG TCC ATC GCC AGT TAT (R) GGG AGC CAC ACT TCA CCA T	96° C 3 min, 95° C 1 min, <u>55C° 1 min</u>, 72C° 1 min, <u>35 times to 2</u>, 4C°	17
3β-HSD typ 1	(F) GGAATCTGAAAAACGGCGGC (R) CTGAGATAAGTAGAACTGTCCTCGGATG	95C° 15min, 94C° 30 sec, <u>61C 40 sec</u>, 72°C 1 min, <u>45times to 2</u>, 72°C10 min, 4°C	19
3β-HSD typ 2	(F) CGGCTAATGGGTGGAATCTA (R) GATCTCGCTGAGCCTTCTTG	95°C 10 min, 94°C 1 min, <u>60 1 min</u>, 72°C 1 min, <u>35 times to 2</u>, 72°C10 min, 4°C	21

Table 2. Primers for different genes and their number of base pairs of PCR fragments.

Attraction genes	Number of base pairs	Positive Control	Reference
17β-HSD typ 5	112	DU 145 (prostate cancer cell lines)	13
CYP19A1	200	DU 145	14
CYP17A1	500	human liver cells	14
CYP17A1	120	human liver cells	14
ER-β	395	human liver cells	17
ER-α	309	ovary, placenta and testis	18
ER-α	108	ovary, placenta and testis	18
3β-HSD typ 1	251	DU145,ovary, placenta and testis	19
3β-HSD typ 1	525	DU145,ovary, placenta and testis	19
3β-HSD typ 2	510	DU145	20

Results

A ladder (DNA mass ladder) used to estimate the PCR fragment size. This ladder consists of a mixture of fragments with sizes of 2000 1800, 1000, 800, 400, 200 and 100 bp.

17β-HSD type 5

17β-HSD primers and the positive control RNA from DU145 cells was used. The expected size of the PCR product with these primers 17β-HSD was 112 bp. Results of the positive control, RNA from DU145 cells, gave us a clear ribbon, while the negative control gave no bands. Results of 17β-HSD primers and RNA from SH-SY5Y cells gave us a clear band with fragments of a size of 112 bp. Programs and primers used are shown in the table and figure1.
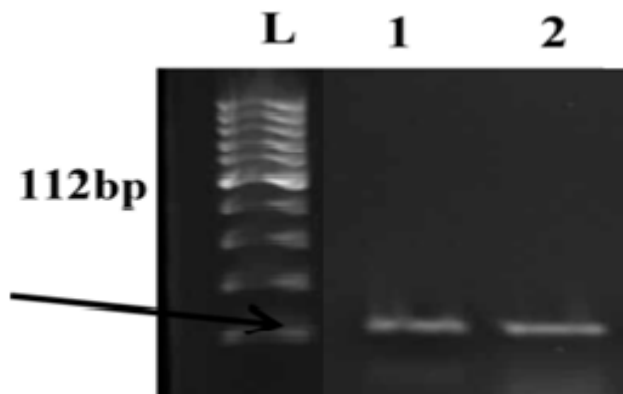
Figure 1. PCR experiments with primers for the 17β-HSD gene. L, ladder (DNA mass ladder); 1, positive control RNA / cDNA from DU145 cells; 2, RNA / cDNA from SH-SY5Y cells.

CYP19A1:

CYP19A1 primers and the positive control with RNA from DU145 cells was used. The expected size of the PCR product with these primers CYP19A1 was 200 bp. Results of the positive control, RNA from DU145 cells, gave us a clear band. As negative control, a mixture of PCR reaction containing no cDNA.

The negative control did not give any band. Results of CYP19A1 primers and RNA from SH-SY5Y cells gave us a clear band with fragments of a size of 200 bp. Programs and primers used are shown in the table1 and fig.2.
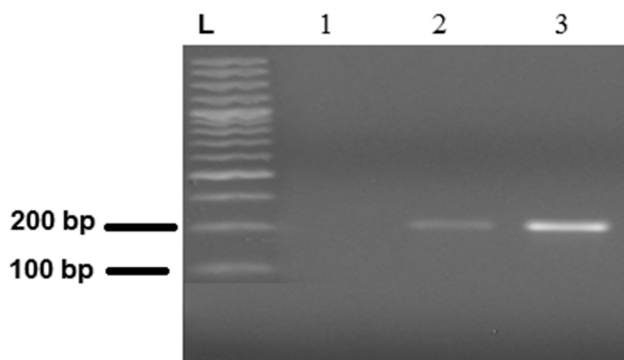

Figure 2. PCR experiments with primers for the CYP19A1 gene. L, ladder (DNA mass ladder); 2, positive control RNA / cDNA from DU145 cells; 1, negative control (no RNA / cDNA); 3, RNA / cDNA from SH-SY5Y cells.

ERβ gene:

ERβ- primers and the positive control with the RNA from human liver cells is used. The expected size of the PCR product with these primers ERβ was 395 bp. As a positive control primers were used to ERβ- RNA from human liver cells. The results of the positive controls, RNA from human liver cells, shows a clear band of the correct size. The result for ERβ primers and RNA from SH-SY5Y cells show a clear band with fragments of a size of 395 bp. The negative control did not give any band.(tab1 and fig.3).
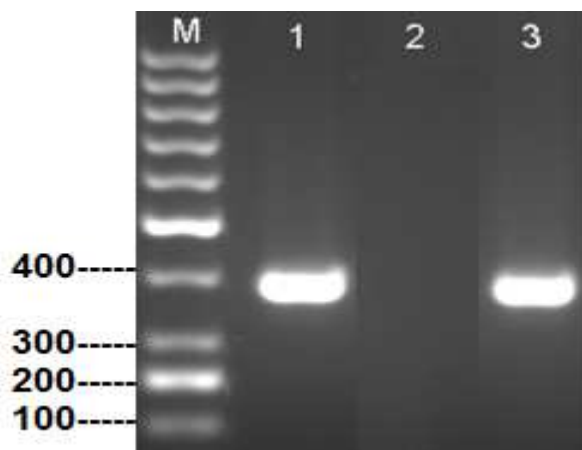

Figure 3. PCR experiments with primers for ER-β gene. M, ladder (DNA mass ladder); 1, positive control RNA / cDNA from human liver cells; 2, negative control (no RNA / cDNA); 3, RNA / cDNA from SH-SY5Y cells.

Discussion

Previous studies have shown that the effects on cholesterol and steroid hormones related to certain neurological diseases. The human SH-SY5Y cell line used in this study, these cells have the characteristics corresponding to neurons in the human brain. The results of this study suggest that some genes (CYP19A1, ER-β, 17β-HSD) is expressed in these neurons, while genes that are not expressed in these neurons is ER-α, 3β-HSD type 1, 3β-HSD type 2; CYP11A1 and CYP17A1.

PCR and electrophoresis was simple and good methods, but we need more studies to ensure the performance of certain genes which results in the present study is uncertain.

Some genes in the study showed clear bands such as ACAT primer, but the interpretation of the results was that they expressed. The results are difficult to interpret, this is especially because of the study's small size and the limited time frame during which the experiments took place. ER-α primers and the positive control was tested several times because they did not give any band in SH-SY5Y cells. The positive control did not work on either of the two primer pairs used and the need for more experiments to test them again by changing the number of cycles or find the optimal annealing temperature to get the band on the positive control. This study could not fully analyze gene expression in the course of the three months during

which the experiments were conducted. Neuroblastoma cells SH-SY5Y, finally, seems to be a suitable cell model for the study of drug effects on the formation and metabolism of cholesterol and steroid hormones.

Conclusion:

One conclusion that can be drawn from the results of this study is to neuroblastoma cell line SH-SY5Y may constitute a possible cell model for future research on hormonal effects and the effect of various drugs on the formation of neurosteroids that influence brain functions.

References:

[1] http://www.alzheimersinfo.se/om-alzheimer/ 2012-11-08

[2] Jin Park, Y., Jang, Y., and Hye Kwon, Y., 2008. "Isoflavones Prevent Endoplasmic Reticulum Stress Mediated Neuronal Degeneration by Inhibiting Tau Hyperphosphorylation in SH-SY5Y Cells" **medicinal food** 2 (3), 528-535.

[3] William., R. Markesbery, M.D. (1997). "oxidative stress HYPOTHESIS IN ALZHEIMER'S DISEASE ". **Free Radical Biology & Medicine**, 23, No. 1, pp. 134-147, 1997.

[4] Lökk, J.,(2006) 'dementia -friendly, intractable complication of Parkinson' **medical Journal. 7** .103rd

[5] Gao, J., Sun, H., Zhu, Z. Ding, Z., And Zhu, L., (2005). "Antioxidant Effects of Dehydroepiandrosterone Are Related to Up-regulation of thioredoxin in SH-SY5Y Cells" , **in Acta Biochimica et Biophysica Sinica**, vol. 37 (2), 119-125.

[6] Azcoitia, J. Yague., G., and L.M Segura., (2011). "Estradiol synthesis within the human brain". **Neuroscience**. Vol 191: 139-147.

[7] Payne, A. H., Hales, D. B. (2004). Steroidogenic Enzymes in the Pathway from Cholesterol to Active Steroid Hormones. **Endocrine Reviews**. 25: 947-970.

[8] J. Lundqvist, Norlin, M., Wikvall, K. 1a,(2010). 25-dihydroxyvitamin D3 Affects hormone production and expression of Steroidogenic enzymes in human adrenocortical NCI-H295R cell. **Biochimica et Biophysica Acta (BBA) -Molecular and Cell Biology of Lipids** 1801: 1056-1062.

[9] Fujimoto J, Sakaguchi A., Hiroshi IA, Teruhiko Tamaya T., (2002) ,. Clinical Implications of the Expression of Estrogen The receptor and -SS in Primary and Metastatic Lesions of Uterine Endometrial Cancers. **Oncology** 2002; 62: 269-277.

[10] R. Guennounl, M.Schumacherl, F. Robert. Delespierrel, M.Gouezou, B. Eychennel, Y. Akwal, Robel P. and E. E. Baulieu. (1997). Neurosteroids: Expression of Functional 3P-hydroxysteroid. Dehydrogenase by Rat Sensory Neurons and Schwann Cells. **European Journal of Neuroscience**, Vol. 9. 2236-2247.

[11] Abd-Elaziz M., Moriya., Akahira JI., T. Suzuki, H. (2005). Sasano Star and progesterone producing enzymes (3β- hydroxysteroid dehydrogenase and colesterol side-chain cleavage cytokrome (P450) in human epithelial ovarian carcinoma: immunohistochemical and real -time PCR studies. **Cancer Sci** .96: 232-239.

[12] OLOV, W,. (2006).New anti-atherosclerosis tested Worrying findings with ACAT inhibitors, which seems to have proaterogena effects. **Medical journal** . 43 Vol. 103. 3270.

[13] Suzuki, K., Nishiyama, T., Hara, N, Ymana, 1K., Takahashi, K., and Labri, F., (2007). Impotance of the intracrine adrena metabolism of androgens in androgen-dependent prostate cancer . **Prostate Cancer and Prostatic Diseases** 10.301-306

[14] Diaz-Cru, E. S., Shapir, C. L., Brueggemeier R.W. (2005). Cyclooxgenase inhibitors suppress aromatase expression and activity in breast cancer cells. **The Journal of Clinical Endocrinology & Metabolism**. 90: 2563-2570.

[15] J. Lundqvist, M. Norlin, Wikvall, K. 1a,(2010). 25-dihydroxyvitamin D3 Affects hormone production and expression of Steroidogenic enzymes in human adrenocortical NCI-H295R cells. **Biochimica et Biophysica Acta (BBA) -Molecular and Cell Biology of Lipids** 1801: 1056-1062, .

[16] Severi, G. Hayes, World Cup, Tesoriero, AA, Southey, MC, Hoang, HN, Padilla, EJD, Morris, HA, English, DR, Sutherland, RL, Boyle, P., Hopper JL And Giles , GG (2008), the Rs 743,572 commom variant in the promoter of CYP17A1 is not Associated with prostate cancer risk or circulating hormonal levels. **BJU International**, 101: 492-496.

[17] Friedman et al.,(2004). Estrogen receptor B is expressed in human embryonic brain cells and is regulated by 17β-estradiol. **European Journal of Neuroscience** 20: 2345-2354.

[18] Dieudonne MN, Leneveu MC, Giudicelli Y, Pecquery R. (2004). Evidence for functional estrogen receptors alpha and beta in human adipose cells: regional specificities and regulation by estrogens. **AM J Physiol**. Vol; 286 (3), s655-61.

[19] Hammer F., Drescher, D. G., Schneider, S. B., Quinkler, M., Stewart, P. M., Aloolio, B., Arlt, W. (2005). Sex steroid Metabloism in Human Peripheral Blood Mono Nuclear Cells Changes with Aging**. J Clin Endocrinol Metab**, 90, 6283-6289.

[20] Magoffin AD, Weitsman SR., (1993) Insulin-like growth factor-1 Stimulates the expression of 3 beta-hydroxysteroid dehydrogenase messenger ribonucleic the ovarian theca-interstitial cells. **Biol Reprod**. v; 48 (5), pp 1166-73.

[21] Asif AR, M. Ljubojevic, Sabolic I Shnitsar V., Metten M., N. Anzai, Muller GA, Burckhardt G., Y. Hagos (2006) Regulation of steroid hormone biosynthesis enzymes and organic anion transported by forskolin and DHEA-A treatment in adrenocotical cell**. Am Physiol Endocrinol Metab**, 291, 1351-1359.

[22] F. Caballero, A. Fernandez, De Lacy AM., Fernandez-Checa JC., Caballeria J., Garcia-Ruiz, C., (2009), Enhanced free cholesterol, SREBP-2 and StAR expression in human NASH. **Journal of Hepatology** 50: 789-796.

[23] P. Brozic , Lanisnik Risner T, S.Gobec,(2008), Inhibitors of 17beta-hydroxysteroid dehydrogenase type 1, **Curr Med Chem**. 15(2):137-50.