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عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	تأثير الميلاتونين وفيتامين (د) على التعبير الجيني للكل-2 و باكس في خط خلية سرطان الثدي.
Title of the proposed project in English	Effect of melatonin and Vitamin D on the gene expression of Bcl-2 and Bax in breast cancer cell line.
المشرف الرئيس - PI	Dr Samina Hyder Haq
التخصص الدقيق للمشرف الرئيس - Specialty of PI	Tissue and cell culture
المشرف المساعد - Co-PI	Dr Abir Alamro
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات - (العليا) بالشهور - Expected time in month to finish	12 months
Abstract of the proposal (No more than 200 words)	Cancer is the main health problem of the world and breast cancer is the most common form of cancer in women. It was ranked as number one among females accounting about 27.4% of all diagnosed female cancer (5378) in the year 2010 [1]. Among Saudi patients, there was a significant increase in the number of cases of breast cancer, which occurs at an earlier age than in western

countries. About 15% of the breast cancers are triple-negative. Apoptosis regulates normal growth, homeostasis, development, embryogenesis and appropriate strategy to treat cancer. Bax is a protein pro-apoptotic enhancer of apoptosis in contrast to Bcl-2 with anti-apoptotic properties. Melatonin is the hormone produced by pineal gland, which is thought to control the sleep is directly related to circadian activity through supra-chiasmatic nucleus. In the last few years a number of studies related to disruption of melatonin's circadian profile by exposure to light at night time has been described to play an important role in initiation, promotion and progression of breast cancer [2,3,4]. Research suggests also that women with low levels of vitamin D have a higher risk of breast cancer. Vitamin D may play a role in controlling normal breast cell growth and may be able to stop breast cancer cells from growing. The study by Imtiaz ., S et al (2012)[5] showed a strong correlation between the vitamin D deficiency and the prevalence of breast cancer in the females. The exact relationship of the effect of melatonin and vitamin D at the genetic and molecular level are still unknown. There is a need to explore the full therapeutic potential of these sunshine hormones on the pathophysiology of breast cancer is worth pursuing.

The aim of the present study is to determine the apoptotic effect of melatonin and vitamin D in breast cancer lines. so we decided to look at two genes related to apoptosis that are Bcl-2 and Bax. The relative expression of these genes would unravel the molecular and genetic pathways that results in apoptosis. We will also study the effect on different caspases activity to measure the rate of apoptosis.

Hypothesis of the proposal

Melatonin and vitamin D have been found to have a therapeutic potential to treat breast cancer and the proposed study will be carried out to unravel the molecular and genetic pathways related to apoptosis of the tumor cells.

Specific objectives

To equip postgraduate students with the latest cell culture and novel research technologies so they compete at the international levels
To teach and train the post graduate students with real hands on training with latest instruments such as LI-COR and tissue and cell culture facility
To develop our research laboratory for the training of our masters and postgraduate students.

Methodology &

Breast cancer line MCF-7 and MDM cultures cells will be set up as follows

Major Techniques to be used	<ol style="list-style-type: none"> 1. Control cultures without any treatment 2. Cultures treated with different doses of melatonin (10, 50,100ng/ml) and 2.5µg/ml of D3). 3. cultures treated with melatonin alone 4. cultures treated with vitamin D alone <p>Following assessment parameters will be employed..</p> <ul style="list-style-type: none"> • To assess cell viability by trypan blue method. • To assess the LDH activity in the different cultures.(by kit) • to calculate cell proliferation by MTT assay.(by kit) • Quantitative evaluation of Gene expression of Bcl-2 and Bax by RTPCR in the control and treated cultures. <ul style="list-style-type: none"> • Assessment of Caspase activity in different treatment groups.(by kit) • Quantitative evaluation of Protein expression of Bcl-2 and Bax by western blot and by in cell –western using Li-COR odyssey machine. • Statistical analysis of the results.
Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Not needed
Recent References	<ol style="list-style-type: none"> 1. Saggi S, Rehman H, Abbas ZK, Ansari AA. Recent incidence and descriptive epidemiological survey of breast cancer in Saudi Arabia. Saudi Medical Journal. 2015;36(10):1176-1180. doi:10.15537/smj.2015.10.12268. 2. Touitou Y, Reinberg A, Touitou D. Association between light at night,

- melatonin secretion, sleep deprivation, and the internal clock: Health impacts and mechanisms of circadian disruption. *Life Sci.* 2017 Mar 15;173:94-106. doi: 10.1016/j.lfs.2017.02.008. Epub 2017 Feb 16. Review. PubMed PMID: 28214594.
3. Van Dycke KC, Rodenburg W, van Oostrom CT, van Kerkhof LW, Pennings JL, Roenneberg T, van Steeg H, van der Horst GT. Chronically Alternating Light Cycles Increase Breast Cancer Risk in Mice. *Curr Biol.* 2015 Jul 20;25(14):1932-7. doi: 10.1016/j.cub.2015.06.012
4. Shiu SY, Leung WY, Tam CW, Liu VW, Yao KM. Melatonin MT1 receptor-induced transcriptional up-regulation of p27(Kip1) in prostate cancer antiproliferation is mediated via inhibition of constitutively active nuclear factor kappa B (NF- κ B): Potential implications on prostate cancer chemoprevention and therapy. *J Pineal Res.* 2013;54:69-79
5. Imtiaz S, Siddiqui N, Raza SA, Loya A, Muhammad A. Vitamin D deficiency in newly diagnosed breast cancer patients. *Indian Journal of Endocrinology and Metabolism.* 2012;16(3):409-413. doi:10.4103/2230-8210.95684.
6. Edwin O. Quisbert -Valenzuela¹ and Gloria M. Calaf¹. Apoptotic effect of nescapine in breast cancer cell lines. *International journal of oncology* 4 2666 8: 2666-2674, 2016DOI: 10.3892/ijo.2016.3476