Asian Journal of Modern and Ayurvedic Medical Science |

ISSN 2279-0772 [ONLINE]

Volume: Volume 4 ,Number 1 |Publication Date: Thursday, January 01, 2015

Published by Mpasvo [article url http://www.ajmams.com/viewpaper.aspx?pcode=8ffd3a7d-6a03-42f6-bb5e-7e9117ced357

PUBLISHED PAPER'S TITLE : *A REVIEW ON PROSTAGLANDINS IN RELATION TO PAINFUL MENSUS*

AND ITS EVALUATION WITH COX-2 ANTIBODY

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Asian Journal of Modern and Ayurvedic Medical Science (ISSN 2279-0772) Vol.4,no.1, January- June 2015.

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Review Paper

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A REVIEW ON PROSTAGLANDINS IN RELATION TO PAINFUL MENSUS AND ITS EVALUATION WITH COX-2 ANTIBODY

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Declaration

The Declaration of the author for publication of Research Paper in Asian Journal of Modern and Ayurvedic Medical Science (ISSN 2279-0772) We Pathak Meenakshi S.N* Prof.Manjari Dwivedi[1] Prof. P.L Pakrasi[2] Awadhesh Kr. Pandey[3]the authors of the research paper entitled ***A REVIEW ON PROSTAGLANDINS IN RELATION TO PAINFUL MENSUS AND ITS EVALUATION WITH COX-2 ANTIBODY*** declare that ,we take the responsibility of the content and material of my paper as we ourself have written it and also have read the manuscript of our paper carefully. Also, we hereby give our consent to publish our paper in ajmams , This research paper is our original work and no part of it or it's similar version is published or has been sent for publication anywhere else.we authorise the Editorial Board of the Journal to modify and edit the manuscript. we also give our consent to the publisher of ajmams to own the copyright of our research paper.

Received November 10, 2014;Accepted November 15, 2014 ,Published January 1,2015

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ABSTRACT: Background:

Pain is such an alarming symptom that it cannot be neglected at all. In females after puberty, pain is mostly found related to Gynaecological disorders. Amongst them the most commonly met across is painful menses. It restricts a woman from her regular routine and confines her to bed.

Prostaglandins, the causative agent of pain, are found in most tissues and organs. They are produced by almost all nucleated cells. They are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. Prostaglandins play a key role in the generation of the inflammatory response. Their biosynthesis is significantly increased in inflamed tissue and they contribute to the development of the cardinal signs of acute inflammation leading to pain.

Objective:

Excess Prostaglandins PGF2α and PGE2 are released from secretory endometrium of patients during painful menses. These cause spasm of uterine muscles during menses, leading to ischeamic pain. The objective of this paper is to find out the exact relation of Prostaglandins with painful menses and to discuss methods for assessment of

Prostaglandin in Endometrium of patients having painful menses,through histology , Immunohistochemistry and Western blot technique.

Keywords: Prostaglandins, pain, Endometrium, histopathology, COX-2, Immunohistochemistry

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INTRODUCTION

Normal menstruation should not be associated with any sort of discomforts such as pain, cramps,and burning sensation. Hence painful menstruation is a variation from normalcy, which needs medical attention. Painful menses are generally perceived as only minor health concerns and thus irrelevant to the public health programme. Data on the frequency of menstrual dysfunction and its impact on health status, quality of life and social integration among women in developing countries are limited. .

Painful menses refers to the occurrence of menstrual cramps of uterine origin. It is a common gynaecological condition with considerable morbidity. The cause of pain i.e. Prostaglandins, their synthesis, metabolism and action on uterus is an important phenomena to understand painful menses.

COX-2 is the dominant source of prostaglandin formation in inflammation. Through Immunohistchemistry we can evaluate that there is decrease in COX-2 expression after treatment or the condition is same,and semiquantitative analysis of COX-2 was done by Western blot technique.

Synthesis of Prostaglandin and its relation to inflammation

Prostaglandins and thromboxane A_2 (TXA_2) termed prostanoids, are formed when arachidonic acid (AA), a 20-carbon unsaturated fatty acid, is released from the plasma membrane by phospholipases (PLAs) and metabolized by the sequential actions of prostaglandin G/H synthase, or cyclooxygenase (COX), and respective synthases.

Prostaglandin production depends on the activity of prostaglandin G/H synthases, colloquially known as COXs, bifunctional enzymes that contain both cyclooxygenase and peroxidase activity and which exist as distinct isoforms referred to as COX-1 and COX-2.

COX-1 subserve housekeeping functions, such as gastric epithelial cytoprotection and homeostasis. COX-2, induced by inflammatory stimuli, hormones and growth factors, so COX-2 appears to be the dominant source of prostaglandin formation in inflammation.

Inflammation and Prostaglandin E_2

PGE_2 is one of the most abundant PGs produced in the body and exhibits versatile biological activities. Dysregulated PGE_2 synthesis or degradation has been associated with a wide range of pathological conditions. Redness and edema result from increased blood flow into the inflamed tissue through PGE_2-mediated augmentation of arterial dilatation and increased microvascular permeability. Pain results from the action of PGE_2 on peripheral sensory neurons and on central sites within the spinal cord and the brain.

Inflammation and Prostaglandin $F_{2\alpha}$

$PGF_{2\alpha}$, derived from COX pathway in the female reproductive system, plays

an important role in ovulation, contraction of uterine smooth muscle and initiation of parturition.

Inflammation and Thromboxane

Thromboxane activation mediates several physiological and pathophysiological responses, including platelet adhesion and aggregation, smooth muscle contraction and proliferation, and activation of endothelial inflammatory responses.

Role of Prostaglandins in Painful menses

Menstruation exhibits many of the classic hallmarks of inflammation. The withdrawal of progesterone in the late secretory phase of the cycle triggers a cascade of inflammatory mediators, leading to a dramatic influx of leukocytes into the premenstrual endometrium. After shedding, the human endometrium exhibits a remarkable and immediate regenerative capacity. This cyclical injury and repair is tightly controlled and, unlike resolution of inflammation at other sites in the body, does not involve loss of function or scarring. Aberrations may lead to menstrual disorders including irregular menstrual bleeding and painful menses.

Progesterone withdrawal during the late secretory phase of the menstrual cycle results in up-regulation of COX-2, an enzyme responsible for prostaglandin synthesis.$PGF_{2\alpha}$ is a potent vasoconstrictor. Premenstrual increases in $PGF_{2\alpha}$ and other vasoconstrictors such as endothelin-1 result in constriction of spiral arterioles. This causes a transient episode of hypoxia in the functional layer of the endometrium. $PGF_{2\alpha}$ induces myometrial contractions and vasoconstriction of the endometrial spiral arterioles.

In 1957, Pickles proposed that a substance produced by the menstruating uterus might be responsible for stimulation of uterine contractility and that this "menstrual stimulant" might be responsible for primary dysmenorrhea. Pickles observed that menstrual fluid of patients with primary dysmenorrhea had higher $PGF_{2\alpha}$ concentration than that of nondysmenorrheic patients and that these patients had an increased PGF-to-PGE ratio. This study was later confirmed by Lundstrom and Green and substantiated by the observation that long-term infusions of $PGF_{2\alpha}$ resulted in symptoms similar to dysmenorrhea.

Csapo and associates measured the intrauterine pressures during menstruation in healthy and dysmenorrheic patients. In contrast to the normal resting pressure of 10 mmHg, the dysmenorrheic patient had levels of 60 mmHg. This increased tone could result in vascular compression and, thus, reduced uterine blood flow. This would further compromise a uterine circulation already reduced by the direct action of $PGF_{2\alpha}$ on blood vessels in the uterus and endometrium. It is this reduced blood flow and resultant anoxia of uterine tissue that is believed to produce the pain of dysmenorrhea.

Uterine Blood Flow and Prostaglandins

Prostaglandins can affect uterine blood flow in three different ways. First, prostaglandins can have a direct effect on the vascular smooth muscle, acting through receptors to produce either uterine vasodilation or vasoconstriction. Second, prostaglandins can increase uterine tone or contractile activity, resulting in a significant reduction in uterine blood flow by mechanical compression of the uterine vasculature. The third potential mechanism is by potentiating or by depressing adrenergic neurotransmission. This is accomplished by altering the local neurogenic release of norepinephrine from the presynaptic nerve terminal or by modifying the responsiveness of the postsynaptic nerve terminal to norepinephrine.

Procedure for Assessment of Endometrial Prostaglandin in Human Endometrium:

1. Histopathological changes in Human Endometrium

2. Immunohistochemistry of Human Endometrium with COX-2 Antibody

3. Western blot analysis of Human endometrium

Plan for Endometrial Biopsy

A woman reporting painful menstrual cycles and had not taken exogenous hormones or used an intrauterine device during the last 3 months should be selected for endometrial biopsy. After proper counselling and written consent of patient, Premenstrual Endometrial Biopsy should be done in secretory phase of menstrual cycle. The sample should be preserved in Bouin's solution.

1. Histopathology

The tissue needed for histopathology should be preserved inBouin's solution. To remove the excess formalin, the tissue is washed in running water, put in cassettes- into a bucket and run in a histokinate for 18hrs for dehydration.The tissue is then placed in wax – in an incubator for about 2hrs.It is taken in an L-shape containing wax and then left for hardening.Once the wax gets hardened, excess wax is cut off and blocks are prepared.The blocks are then finely cut in a microtome , put to a water bath and 2-3 micron section is put on albumin coated slides and re incubated for about 1 hr at 60^0C or overnight at 37^0C. These slides are then processed with H&E Stain.

Method of H&E Stain:

- The slides are 1st treated with Xylene for 10-15 min for dewaxination. To remove excess Xylene, it is then blotted with blotting paper.
- It is then treated with alcohol for 2-5 min and then kept in running water so that excessive water gets dissolved.
- The slides are then treated with Haematoxylin stain for 3-5 min which gives the stain a red colour.Then kept in running water until the red colour became blue.
- The slides are then treated with acid(1% acid HCl) for 2-5 min-Here the nuclei takes the blue stain and cytoplasm red.The slides are then kept under running water for 10 min.
- Slides are dipped in Eosin 1% for few seconds.
- Slides are then dehydrated with graded alcohol- 90, 95, 100% for a few seconds, blotted and then mounted.
- Now the slides are ready to be visualized under a microscope-for histology .

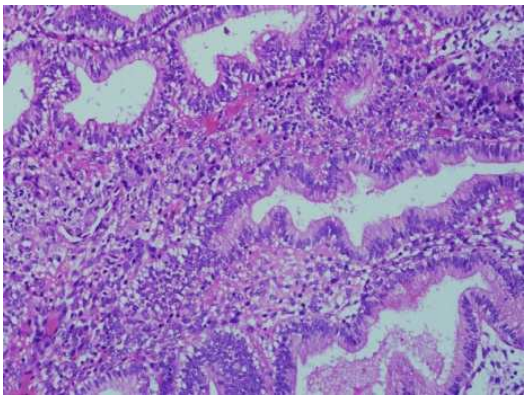

Fig.1

In this way histology of endometrium is visualized. In

fig. 1 there are inflammatory mediators seen in stroma of endometrium. The stromal cells have vesicular nuclei with occasional prominent nucleoli, abundant cytoplasm.

2. Procedure for Expression of COX-2 in Human Endometrium through Immunohistochemistry:

- Cut sections of 2-3μ from endometrial tissue block are taken on Poly-L-Lysine coated slides and the slides are incubated at 62-65°C for 1 hour.
- Slides are deparaffinized in Xylene and hydrated in descending grades of ethanol.
- Slides should be washed in Phosphate Buffer Solution twice (5 min each) and kept under running water for 10 min.
- Slides are then placed in plastic Coplin jar filled with 10mm Citrate Buffer (pH 6.0) –Antigen Retrieval. Slides are cooled at room temperature and washed with PBS(Ph 7.6), 3 times each for 5 min.
- Slides are incubated for 2 min. in 0.3% H2O2 in methanol for endogenous peroxidase blocking and slides are again washed with PBS and incubated in horse serum for blocking non-specific sites.
- Later Polyclonal COX2 antibody should be added and slides should be kept for overnight at 4°C. Slides are again washed with PBS and incubated with universal secondary antibody for 30 min.
- Slides should be re- washed in PBS and are incubated with C-Complex (ABC kit)
- Slides are washed again in PBS and are incubated with DAB for colour foramation.
- Slides should be then dipped in Distilled water to stop reaction and Counterstained with Harris's Haematoxylin for 1 min.
- Slides are kept in running water for 5 min and are rehydrated in graded alcohol and cleared in Xylene.
- Slides are then mounted with DPX. The Brown ppt. on tissue represents our desired antigen (COX-2).

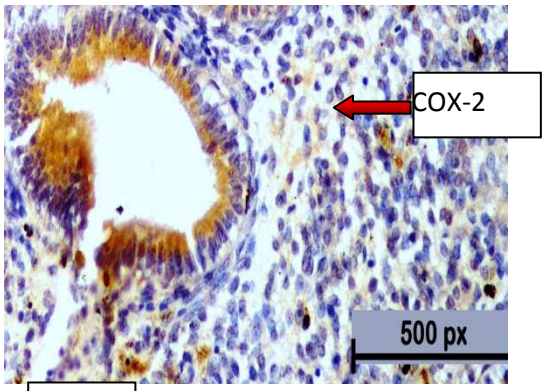

Fig.2

In fig.2, COX-2 expression is clearly seen in the glandular epithelium denoted by brown precipitate.

3. Western blot analysis of Human endometrium

The semi-quantitative analysis of Prostaglandins in Human Endometrium can be done through Western blot method.

Semi Quantitative analysis by Western Blotting Method (John G. Edward et. al., 2002 American Association for Cancer, *Vol. 8, 1857–1862, June 2002)***.**

Steps for Western Blot:

1) Frozen samples should be homogenized mechanically in a buffer [150 mM sodium chloride, 0.1 M Tris (pH 8), 1% Tween-20, 50 mM diethyldithiocarbamic acid,
2) 1 mM EDTA pH 8 (Sigma)] containing protease inhibitors, before sonication and centrifugation at 4°C for 3 min. A Bradford assay (Bio-Rad, Hemel Hempstead, United Kingdom) is used to determine the protein concentration of each supernatant.
3) Samples are loaded into a 10% SDS-PAGE, to give 150 g protein/well. Once through the stacking gel, proteins are resolved at 150 V for 4 h.

TEMED(
Tetramethylethylenediamine)

TEMED is used with ammonium persulfate to catalyze the polymerization of acrylamide when making polyacrylamide gels, used in gel electrophoresis, for the separation of proteins or nucleic acids.

1X Tris-glycine-SDS Buffer (10X buffer diluted to 1X concentration prior use)

4) Transfer to a nitrocellulose membrane(Hypobond-ECL; Amersham Pharmacia Biotech, Amersham,United Kingdom) i performed by semi-dry electroblotting (Bio-Rad).
5) Complete protein transfer is confirmed by staining both gel and membrane with Ponceau S (81462; Sigma).
6) The blotted nitrocellulose membrane is then blocked in 10% milk/Trisbuffered saline wash buffer [0.05 M Tris (pH 7.5), 0.15 M sodium chloride, 0.1% Tween-20 (Sigma)] overnight at 4°C.
7) The membrane is probed with a monoclonal COX-2 primary antibody (SC-1745; Santa Cruz Biotechnology/Autogen Bioclear) for 90 min at room temperature.
8) After washing, the membrane is incubated with a horseradish peroxidise conjugated secondary antibody (SC-2020; Santa Cruz Biotechnology)for 1 h at room temperature.
9) The membrane s washed and developed using ECL(Emitter-coupled logic)following the manufacturer's protocol.
10) The membrane is exposed to Hyperfilm for between 2 and 20 min.
11) Developed films are scanned and band densitometry calculated on an densitometer .
12) Background activity, the mean value of the perimeter of each template cell, is subtracted from each sample.
13) Four samples are chosen in addition to the COX-2 standard (NP04;) foruse as internal positive controls and run on each gel.
14) All of the gels are run under identical conditions with the same batch of reagents and densitometry results standardized between gels.
15) COX-2 densitometry results are also normalized to membranes stripped and reprobed for tubulin (T-9026 primary antibody (Sigma) and P0260 secondary antibodies.
16) The semiquantitative nature of this protocol is validated by densitometric analysis of serial dilutions of the two samples with the strongest COX-2 bands.
17) The specificity of the antibody is confirmed by preabsorbing the COX-2 primary antibody with a COX-2 blocking peptide overnight at 4°C.

In this way a semi-quantitative analysis of COX-2 can be done to make the experiment more efficient statistically.

CONCLUSION

From the present study we come to know that Prostaglandins are the main cause for painful menses. Hence there is a relation in painful mensus and Prostaglandin increase and through Histopathology, Immunohistochemistry of COX-2 of Human Endometrium and semi quantitative analysis of COX-2 by western blot, we will be able to diagnose the post treatment evaluation of efficacy of treatment.

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