

Sialic Acid Is A Novel Biochemical Marker In Sera Of Iraqi Endometriotic Patients¹.

Rasha Z. Jasim¹ Bushra H. Ali² Zohair I. Al-Mashhadany²

1. PhD.student of biochemistry, Department of chemistry /College of education for pure sciences (Ibn Al-Haitham). Baghdad University.

* E-mail of the corresponding author biochemistry_girl83@yahoo.com

2. PhD. of biochemistry, Department of chemistry / College of education for pure sciences (Ibn Al-Haitham). Baghdad University.

Abstract

Sialic acids are nine carbon acidic sugars typically found as the terminal residue of cell surface sugar chains as well as on secreted glycoproteins and in the extracellular matrix. Structural diversity and unique strategic location of sialic acids (Sias) on the cells make them one of the most important molecules in life and set the challenges for sialoglycobiologist. An important facet of Sia biology is the function of Sia-binding immunoglobulin-like lectins (Siglecs), receptors that are largely expressed across the major leukocyte lineages to mediate important innate and adaptive immune functions.

A postulated autoimmune aetiology of endometriosis derives from reports on increased polyclonal B-cell activity, abnormalities in function of B and T cells, , high B-cell and T-cell counts, and reduced natural-killer-cell activity. Also, high serum concentrations of IgG autoantibodies .Sialic acid binding immunoglobulins (Ig)-like lectins (siglecs) belong to I-type lectin with a selective expression on the haematopoietic cell lineages. These have amazing structural diversity to recognize and interact with an array of linkage-specific sialic acids on a glycan structure express on host cells as well as pathogen. Fourteen human and nine murine siglecs have already been identified and the list is still increasing. Innate immune system is the first line of defense evolved during the years of evolution. This is responsible for controlling and clearing invading pathogens. The results of the present study indicate that Sialic acid is a novel biochemical marker related to the immune pathological alterations of endometriosis as shown in G1 and G3 . Also, the results showed the role of zoladex in alteration immune responses as shown in G2.

Keywords Sialic acid . endometriosis . immunoglobulins . autoimmune diseases.

1.0 Introduction

Endometriosis is a common benign chronic – inflammatory gynecologic disorder (1,2) , defined as the presence and proliferation of functional endometrial glands (endometrial - like tissue) outside of the normal location (uterine cavity) , (1,3,4,5) , mainly causes pain and infertility (6). Endometriosis gets its name from the word endometrium, which is the tissue lining the uterus (womb) (7). The humoral immune response may explain endometriosis in general terms, in accordance with the characteristics of an autoimmune disease(8,9)

Although most women experience retrograde menstruation, which may play a role in the seedling and establishment of implants, few develop endometriosis. Hence , menstrual tissue and endometrium that is refluxed into the peritoneal cavity is usually cleared by immune cells such as macrophages, natural killer (NK) cells, and lymphocytes. For this reason, immune system dysfunction is one likely mechanism for the genesis of endometriosis in the presence of retrograde menstruation (1).

Immune alterations include increased number and activation of macrophages, decreased T cells reactivity and NK cells cytotoxicity, increased circulating antibodies and changes in the cytokine system (12). Natural killer cells are immune cells that have cytotoxic activity against foreign cells. Humoral immunity has also been shown to be altered in affected women and is suggested to play a role in the development of endometriosis. There is substantial evidence that immunologic factors play a role in the pathogenesis of endometriosis and endometriosis-associated infertility. Decreased natural killer cell cytotoxicity leads to an increased likelihood of implantation of endometriotic tissue and modulation of growth and inflammatory behavior of ectopic endometrial implants . Indeed , the immune system of the body consists of two major components. B lymphocytes and T lymphocytes. The B lymphocytes are mainly derived from bone marrow cells in higher animals. The T lymphocytes are of thymic origin. The B cells are responsible of the synthesis of circulating humoral antibodies , also known as immunoglobulins, so Immunoglobulins (Igs) are also called antibodies (Abs).

¹ This paper is a part of PhD. thesis of the first author.

Immunoglobins , glycoproteins that comprise antibody molecules and having patterns of molecular structure (antigenic determinants) , play a major role in the bodys defense mechanisms, and produced in response to foreign substances entering the living body- antigens or immunogens , binding to them and forming antigen-antibody complexes(11).

Sialic acids (Sias) (N-acetyl neuraminic acid) are a diverse family of monoaccharaides. They are unusual sugars with a shared nine-carbon backbone that are widely expressed on the surfaces of all cells in all animals of the deuterostome lineage .Given their remarkable diversity in structure, glycosidic linkage, and underlying glycan chains, as well their exposed location, it is not surprising that Sias have numerous roles in many aspects of immunology (12,13).

In particular , Sialic acid is a marker of the acute-phase response, because many of these glycoproteins have sialic acid as the terminal sugar on their oligosaccharide chain. Serum sialic acid is almost completely (99%) bound to glycoproteins and lipids (13). In this regard , sialic acid binding immunoglobins (Ig)-like lectins (siglec) has a recently been shown to be associated with cell death regulation of myeloid cells. Siglecs play a particular role in cell death regulation under inflammatory conditions. Siglecs contribute to the limitation of innate immune responses by regulating the life span of neutrophils and eosinophils , therefore additional knowledge about the role of siglecs under inflammatory conditions may provide important new insights for the pathogenesis and treatment of infectious and autoimmune diseases (14). Moreover, Several studies have suggested that terminal sialic acid residues on IgG Fc glycans (Fc :- fragment crystallizable region) mediates anti-inflammatory responses (15).

In fact , in chronic and autoimmunity disorders , aberrant glycosylation can be an effective diagnostic and prognostic marker (16).

2.0 Material and method (Experimental)

2.1 Subjects

Seventy five (75) consecutive women patient of reproductive age (25-40) years were enrolled in this study , who attended departments of Gynecology and obstetrics related to the following hospitals : Baghdad teaching hospital / Medical city , Al-Yarmook teaching hospital and Kamal Al-Samarray hospital from April to October 2013. Patients were divided into three groups , Group 1 (G1) included (25) endometriotic patients that are newly diagnosed. Those patients don't administrate any treatment or anti-inflammatory medications. Second group : group 2 (G2) consist of (25) endometriotic patient who treated with zoladex for 3 to 5 months , they received zoladex injection every 28 day after the date of diagnosis. The third group: group 3 (G3) involved (25) patient with recurrent endometriosis , they were post treatment of zoladex and diagnosis revealed recurrence of endometriosis. Patients groups were compared with two control groups , with matched age with Patients' groups . The first control group (C) included (25) healthy women , and the second control group or pathological control group (PC) involved (25) women suffering from infertility caused by gynecological disorders unrelated to endometriosis.

2.2 Blood sampling and Parameters Determination

Five milliliters (5 mL) of venous blood were collected from the all subjects enrolled in this study , placed into plain tubes until coagulation was performed . Serum was separated from blood cells by centrifugation at 4000 r.p.m . The sera obtained and divided into small portions and kept frozen until analysis . Determination of serum Imunnoglobin G (IgG) and Imunnoglobin A (IgA) levels (mg / dL) and Imunnoglobin M (IgM) levels was done by radial immunodiffusion plate, difusing in agrose gelcontaining a specific antibody will form an immuno-complex, visible as a ring around the well. The ring diameter is direct proportional to the concentration of the analyzed protein. The proportion corresponds to the diffusion time. In fact , at the end (72 hours) , the square of the diameter will be in linear proportion to the concentration.

On the other hand , the quantitative sandwich enzyme immunoassay (ELISA) technique was employed for the determination of Sialic acid. Antibody specific for Sia has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Sia present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Sia is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any

unbound avidin-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of Sia bound in the initial step. The color development is stopped and the intensity of the color is measured.

2.3 Statistical Analysis

The results expressed as mean \pm SEM. Students t-test was applied to compare the significance of the difference between all the studied groups. P-value ($p<0.05$) , ($p<0.001$) considered statistically significant and highly significant respectively.

3.0 Results

Table (1) shows the sera levels of some biochemical parameters in the studied groups. The results from this study revealed that IgG levels were highly significant increase ($p<0.001$) in G1 (1660 ± 155) mg /dL compared with group C(1039 ± 42) mg/dL, while no significant increase ($p<0.05$) was observed in G1(1660 ± 155) mg /dL compared with PC group(1428 ± 124) mg/dL , in G2(1282 ± 213) mg/dL compared with C group(1039 ± 42) mg/dL , and in G1 (1660 ± 155) mg/dL compared with G3(1441 ± 78) mg /dL. Also , there was no significant decrease ($p\geq0.05$) in G2(1282 ± 213) mg/dL compared with G1(1660 ± 155) mg /dL.

IgA levels were highly significant increase ($p<0.001$) in G1 (401 ± 16) mg/dL compared with groups C (293 ± 22) mg/dL and PC (305 ± 13) mg/dL, while significant decrease ($p<0.05$) was noticed in G2 (301 ± 42) mg/dL compared with G1(401 ± 16) mg/dL. Also, there were no significant difference ($p\geq0.05$) in G2 (304 ± 42) mg / dL compared with C group(293 ± 22) mg/dL, whereas high significant decrease ($p<0.001$) observed in G3 (349 ± 19) mg/dL compared with G1(401 ± 16) mg/dL.

Our results also implied that IgM levels were highly significant increase ($p<0.001$) in G1 (332 ± 7) mg/dL compared with groups C (140 ± 12) mg / dL and PC (204 ± 56) mg/dL ,while high significant decrease ($p<0.001$) was observed in G2 (136 ± 25) mg/dL compared with G1(332 ± 7) mg/dL. Also there were no significant difference ($p\geq0.05$) in G2(136 ± 25) mg/dL compared with group C (140 ± 12) mg / dL. Moreover , there were high significant decrease ($p<0.001$) in G3(241 ± 15) mg/dL and compared with G1(332 ± 7) mg/dL.

Sia levels were high significant increase ($p<0.001$) and significant increase ($p<0.05$) in G1(43.2 ± 2) μ g/mL compared with groups C(35.5 ± 1) μ g/mL and PC (36.2 ± 1) μ g/mL respectively, while high significant decrease ($p<0.001$) was found in G2(34.8 ± 3) μ g/mL compared with G1(43.2 ± 2) μ g/mL. Also , there were no significant differences ($p\geq0.05$) shown in G2(34.8 ± 3) μ g/mL compared with C(35.5 ± 1) μ g/mL group and in G3(41.3 ± 2) μ g/mL compared with G1(43.2 ± 2) μ g/mL.

Table 1 Levels of Immunoglobulin G , Immunoglobulin A , Immunoglobulin M and sialic acid in sera of the studied groups.

Parameter	Mean \pm SEM C	Mean \pm SEM PC	Mean \pm SEM G1	Mean \pm SEM G2	Mean \pm SEM G3	C vs G1 T.Test	PC vs G1 T.Test	G1 vs G2 T.Test	C vs G2 T.Test	G1vs G3 T.Test
IgG	1039 \pm 42 (mg/dL)	1428 \pm 124 (mg/dL)	1660 \pm 155 (mg/dL)	1282 \pm 213 (mg/dL)	1441 \pm 78 (mg/dL)	H.S	N.S	N.S	N.S	N.S
IgA	293 \pm 22 (mg/dL)	305 \pm 13 (mg/dL)	401 \pm 16 (mg/dL)	301 \pm 42 (mg/dL)	349 \pm 19 (mg/dL)	H.S	H.S	S	N.S	H.S
IgM	140 \pm 12 (mg/dL)	204 \pm 56 (mg/dL)	332 \pm 7 (mg/dL)	136 \pm 25 (mg/dL)	241 \pm 15 (mg/dL)	H.S	H.S	H.S	N.S	H.S
Sia	35.5 \pm 1 (μ g / mL)	36.2 \pm 1 (μ g / mL)	43.2 \pm 2 (μ g / mL)	34.8 \pm 3 (μ g / mL)	41.3 \pm 2 (μ g / mL)	H.S	S	H.S	N.S	N.S

4.0 Discussion

Although the pathogenesis of endometriosis has not been clearly defined, abnormal levels of immune system cells, including macrophages, dendritic cells and natural killer cells, have been observed within the abdominal cavities of patients with endometriosis. These cells, however, are unable to detect and eliminate ectopic endometrial cells. Moreover, immune system cells in the abdominal cavity were found to be dysfunctional (17). The immune system has been shown to play a significant role in the pathogenesis of endometriosis. Based on these recent findings, endometriosis is starting to be treated as an autoimmune disease (18). However, complicated reactions may occur within the abdominal cavity, due to endometriosis-induced secretion or reactions of immunoglobulins and cytokine (17).

Indeed, We have evaluated endometriosis immunologically by analyzing the expression of some biochemical parameters including immunoglobulins and sialic acid. Accumulating evidence suggests that systemic T cell activity influences the pathogenesis of endometriosis. Natural killer (NK) cells are also altered in endometriosis. Sera from women with endometriosis have been shown to reduce (NK) cell activity. This reduction in activity is probably caused by monocyte or macrophage secretions that modulate immune and nonimmune cells. In addition to alterations in T cell function, many recent findings have shown that B-cell function is altered in endometriosis patients as evidenced by abnormal antigen-antibody reaction and increased B cell function. In this regard, anti-endometrial antibody (IgG) has been detected in the sera of endometriosis. (18). Moreover, IgG is primarily involved in chronic inflammation and autoimmune reactions (17). For the reasons mentioned above, IgG level was highly significant elevated in G1 compared with C group. Our results are also in agreement with a recent study which revealed that Autoantibodies against this antigen have been observed in other autoimmune diseases like endometriosis. These results also revealed that IgG were identified in patients with endometriosis when compared with controls. The assay for antibodies against these proteins may be used for diagnosing endometriosis (9).

On the other hand, the non-significant difference in IgG level between G1 and PC reflect that high level of IgG is associated with disorders causing infertility. In this way, a recent study have mentioned that IgG is involved in adaptive immune responses, in patients with and without endometriosis (17).

IgG autoantibodies play a role against endometrial and ovarian tissues and elevate in the sera of affected women (1). In contrast, IgG level was depressed in sera of patients after treatment with zoladex, as shown in G2 compared with G1.

Previous studies support our findings, revealing that GnRH (including zoladex) reduce inflammatory reactions and exerts potent effects on the immune system. Besides, zoladex may reduce adhesions caused by inflammatory responses(19-21). Anyway, these adhesions are associated with moderate and severe cases of endometriosis. (22)

In accordance with (23,24), endometriosis is a recurrent disease. This suggestion confirms our findings related to the non-significant variation of IgG level in G3 compared with G1, revealing that immunological disorders have come back again.

Indeed, when B cells differentiate into plasma cells, the initial immunoglobulins produced include IgA, which is primarily involved in mucosal immunity. Previous findings showing that the concentration of a specific IgG autoantibody was increased in the peritoneal fluid of patients with endometriosis and endometrial glandular epithelial staining for both IgG and IgA was significantly increased, suggests that endometriosis may be an autoimmune disease. Broadly, Immunoglobulins are produced following a process called "class-switching". Thus, various antibodies can be present in the peritoneal cavity in the absence of any exogenous infection (17). These explanations are also agreed with our findings since IgA level was highly significant increase in G1 compared with C group. On the other hand, the high significant increase of IgA in G1 compared with PC group reflect that IgA is a biochemical marker linked to endometriosis regardless of infertility disorder.

In accordance with (17), IgA is primarily involved in mucosal immunity. Hence, immune aspects of endometriosis reveal the of the uterine mucosal immune system (25). Further, IgA have been detected in the sera and vaginal and cervical secretions of endometriosis patients(18).

Otherwise , the significant decrease of IgA in G2 compared with G1 and non- significant variation between G2 and C group highlight the reactive role of zoladex in reducing immunological responses , in part , responsible for the pathogenesis of endometriosis (20) , the high significant decrease in IgA level in G3 compared with G1 reflect that mucosal immunity related to IgA may be less abnormal with recurrent cases, but immune system is still abnormal (1,2).

Immunoglobulins are involved in the adaptive immune response , in patients with endometriosis. Particularly, IgM is primarily involved in acute inflammation (17). Since endometriosis is an inflammatory disease (1,2), IgM level was high significant elevated in G1 compared with C group . Our findings are also in agreement with previous studies , which revealed that high serum concentrations of IgM is associated with autoimmune aetiology of endometriosis (18, 26).

On the other hand , the high significant elevation of IgM level in G1 compared with PC group reveal that high level of IgM may specially associated with endometriosis regardless of general cases of infertility. Endometriosis may cause acute disorders (27) , IgM is primarily involved in acute inflammation (17). Taken together , IgM can be considered a good biochemical marker of endometriosis.

The high significant decreasing of IgM in G2 compared with G1 and the non-significant difference between G2 and C group indicate the crucial role of zoladex in regression the inflammatory nature responsible for adhesions in moderate and sever cases of endometriosis (20). Moreover , zoladex as a GnRH is able to markedly reduce the inflammatory reaction (19).

However, the high significant decrease of IgM level in G3 compared with G1 indicate that acute inflammation linked with IgM may be less sever in recurrent cases but inflammatory disorders are indicated in all cases. (1,2)

Sialic acids are nine carbon acidic sugars typically found as the terminal residue of cell surface sugar chains as well as on secreted glycoproteins and in the extracellular matrix. Structural diversity and unique strategic location of sialic acids (Sias) on the cells make them one of the most important molecules in life and set the challenges for sialoglycobiologist (28). An important facet of Sia biology is the function of Sia-binding immunoglobulin-like lectins (Siglecs), receptors that are largely expressed across the major leukocyte lineages to mediate important innate and adaptive immune functions (29).

Since endometriosis is a chronic – autoimmune disease , (1,9). Consequently , Sialic acid level was high significant elevated in G1 compared with C group. Furthermore, the significant increase in sialic acid in Group1 (G1) compared with PC group supports that infertile women suffering from immunological / inflammatory disorders (30) Indeed , glycans exert their biological influence in three ways. they are targets for recognition by glycan-binding proteins (GBPs). Recognition of glycans by GBPs plays a central role in cellular communication and cell trafficking. These interactions pervade every multicellular system and have been much explored in areas such as immunology (e.g.siglecs) (31). Although siglecs were identified in early eighties, not much work has been done to explore the consequences of sialic acids acquisition by various pathogens and their interactions with immune system in sialic acids-siglec dependent manner in promoting infection or mediating immune activation (28).

In fact , sialic acids function as a recognition sites for various lectins and antibodies, indicating the unique nature of the molecule. Many immunological functions are attributed to sialic acids such as formation of negatively charged barrier for host to reduce interaction with pathogen and dampening the classical or alternative pathways of complement by selective deposition on pathogen surface (28).

In particular , glycosylation can also influence autoimmunity by modulating the activity of key regulatory components. For example, immunoglobulin G (IgG) antibody-mediated inflammation is associated with specific antibody glycoforms These autoantibodies form immune complexes (IC) with self-antigens that in turn drive inflammation via recruitment of complement and effector cells, subsequently leading to localized tissue damage. Specific IgG Fc glycoforms are associated with IC formation, binding of activatory IgG Fc γ -receptors (Fc γ R), complement activation, and severity of inflammatory response in various autoimmune diseases (31).This

inflammation response may cause a defective " immune surveillance " that prevents elimination of the measured debris and promote the implantation and growth of endometrial cells , in the ectopic sites (32).

Conversely , sialic acid level was significantly decreased in G2 compared with Group G1 , these findings reflex the provital role of zoladex which is able to markedly reduce the inflammatory reaction , regress the inflammatory nature responsible for adhesions , and exert strong immunomodulatory effects on the immune system. [18-20]. In this way , the non-significant difference in Sia level between G2 and control C group indicates the biochemical action of zoladex.

Finally, the non-significant difference between G3 and G1 confirms that endometriosis is a recurrent disease (23,24) because immunological and inflammatory responses have appeared again. It is reported that the treatment of a GnRH agonist, significantly suppressed endometriotic implant size but the implant spontaneously returned to pre-treatment size later on in sites where the autotransplantation and immunological dysfunctions took place. This suggests that hormonal treatment does not lead to a complete suppression of endometriotic foci and that recurring lesions appear to grow from the residual loci. It is reported that the treatment of a GnRH agonist, significantly suppressed endometriotic implant size but the implant spontaneously returned to pre-treatment size later on in sites where the autotransplantation and immune/inflammatory responses took place again. Similarly , recurrence after surgery occurs because of in situ regrowth of residual endometriotic lesions or cells not completely removed in the surgery , growth of microscopic endometriosis undetected at surgery or the development of de novo lesions , or a combination of these(33).

5.0 Conclusion

Our study reported for the first time a positive relationship between sialic acid and pathogenesis of endometriosis. This suggest that sialic acid is considered a novel a novel biochemical marker for endometriotic patients (both newly diagnosed G1 and recurrent cases) due to its diagnostic power. Also , the results showed the role of zoladex (as a medical treatment) in alteration immune responses during the period of treatment as shown in all parameters in G2. Moreover , our findings indicated that endometriosis is now considered a chronic inflammatory disease , with inflammation not only limited to peritoneal cavity, but also spread to systemic level as signaled by elevated serum levels of sialic acid and immunoglobulins.

Finally , our observations related to autoimmune disorders to be more common in women with endometriosis supports the possibility that pathogenesis of endometriosis may involve a definitive immune response in these patients , the next few years promise to be exciting period for research to find more biochemical markers linked to immune dysfunctions in endometriotic patients.

Acknowledgments

We are grateful to all the staff of department of Gynecology and obstetrics in Baghdad teaching hospital / Medical city with special gratitude to Dr. Nawfal Fawzi Anwer , Dr. Najah Shaker Yassen , Dr. Saba Riyad Shaker and Dr. Afraa Mahjoob to help in providing the most number of blood samples and for valuable suggestions. Also we thank the chemist Lamya Taha Mohamed in the educational laboratories of medical city to help in saving samples.

References

- (1) Hoffman , B. , Schorge ,J. , Schaffer,J. , Halvorson , L. & Brashaw , K .(2012). Williams Gynecology / Section 1 :-Benign General Gynecology ,Chapter 10:-endometriosis . 2nd edition.
- (2) Arnold , J. , Mechsnar , S. , Schneider , A. & Arellano , M. (2013). Neuroendocrinology and immune function. Journal of Brain , Behavior and Immunity , 29 ,S10-S13.
- (3) Estrade , C. , Arellano , M. , Schneider , A. & Mechsnar , S. (2013). Neuroimmunmodulation in the pathogenesis of endometriosis . Journal of Brain , Behavior and Immunity,29,S2-S9.

- (4) Schweiße , K., Rabe , T. , Langhardt , M. , Woziwodzki , J. , Petraglia , F. & Kiesel , L. (2013) . Endometriosis – Pathogenesis, Diagnosis, and Therapeutic Options for Clinical and Ambulatory Care, Journal of Reproductive Medicine and Endocrinology , 10(1): 102-119.
- (5) Al-Azzawy, M. (2012) . Goserelin acetate for recurrent endometriosis. Journal of Iraqi Medicine Science , 10(1):22-26.
- (6) Pillet , L., Schneider , A., Borghese , B. et al.(2012) Deep infiltrating endometriosis is associated with markedly lower body mass index : a 476 case – control study. Hum Reprod , 27(1):265-72.
- (7) Pollard , J .(2011) . Endometriosis Finding help for pain & pregnancy. J. of Agrilife extension, 15,1-5.
- (8) Podgaec , S. , Junior , J. & Chapron , C.(2010) . Th1 and Th2 immune responses related to pelvic endometriosis. Journal of Rev. Association Medical Bras, 56(1):92-98.
- (9) Suresh,P. , Venkatesh , T. , Rajan , T. & Tsutsumi,R .(2010). Molecular Pathology and therapy of endometriosis : Revisited , J. of Andrology & Gynecology : Current Research .1(3): 1000105.
- (10) Horak , S. & Olejek , A. (2012) . Endometrial tumors in postoperative scar- pathogenesis, Diagnosis and treatment. Endometriosis - Basic Concepts and Current Research Trends, Prof. Koel Chaudhury (Ed.), ISBN: 978-953-51-0524-4, InTech, Available from: <http://www.intechopen.com/books/endometriosis-basic-concepts-and-current-research-trends/endometrial-tumours-in-postoperative-scars-pathogenesis-diagnostics-and-treatment>.
- (11)Kaetzel,C. (2014). Coevolution of Mucosal Immunoglobulins and the Polymeric Immunoglobulin Receptor. Evidence That the Commensal Microbiota Provided the Driving Force. Hindawi Publishing Corporation ISRN Immunology. Article ID 541537, 20 pages. [http:// dx.doi.org/10.1155/2014/541537](http://dx.doi.org/10.1155/2014/541537).
- (12) Varki , A. & Gagneux , p. (2012). Multifarious roles of sialic acids in immunity. J. of New York Academy of Sciences, 1125316-36.
- (13) Balu Mahenderm K. , Gnandesigan , E. , Rekha Kumari , D. et al. (2005) .Evaluation of Sialic acid levels in Patients with type 2 Diabetes Mellitus. IOSR Journal of Dental and Medical Sciences, (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861. 5(1):33-36.
- (14) Gunten, S. & Simon, H. (2006). Sialic acid binding immunoglobulin-like lectins may regulate innate immune responses by modulating the life span of granulocytes. The FASE Journal,20, 601-605.
- (15)Shade, K. & Anthony,R..(2013) Antibody Glycosylation and inflammation. J. of Antibodies,2,392-414.
- (16) Dalziel , M. et al. (2014). Emerging Principles for the Therapeutic Exploitation of Glycosylation. Journal of Science, 343. DOI: 10.1126/science.1235681www.sciencemag.org.
- (17) Yeo,S. , Won,Y. , Lee.,H et al. (2013). Increased expression of Pattern recognition receptors and Nitric oxide synthase in patients with endometriosis. Int. J. Med. Sci, 10(9):1199-1208.
- (18) Bedaiwy , M. & Falcone,T.(2004) . Laboratory testing of endometriosis. J. of Clinica Chimica Acta, 304 ,41-56.
- (19) Khan , K. , Kitajima , M . & Hiraki , K . (2010). Changes in tissue inflammation, angiogenesis and apoptosis in endometriosis, adenomyosis and uterine myoma after GnRH agonist therapy. Journal of Human Reproduction , 25(3):642-653.
- (20) Nirgianakis , K. , Bersinger , N. & Mckinnon , B. (2013). Regression of the inflammatory microenviroment of the peritoneal cavity in women with endometriosis by GnRHa treatment.Eurpian. Journal of Obstet. Gynecol.Reprod. Biol, 170(2):550-4.
- (21) Dixit , V. , Yang , H. , Udhayakumar , V. & Sridaran , R.(2003) . Gonadotropin- Releasing Hormone Alters the T Helper Cytokine Balance in the Pregnant Rat. Journal of Biology and Reproduction, 68: 2215-2221.

- (22) Perloe, M. & Christie, L. (1986). Miracle Babies and other happy Endings for couples with fertility problems / Chapter 17 Endometriosis conquering the silent invader. Copyright © 1986 Associates.
- (23) Muntahana , F. , Jiao , J. & Cuil , B. (2013). A Rare Presentation of Endometriosis with Recurrent Massive Hemorrhagic Ascites which Can Mislead. International Journal of womans Health reproduction Science, 2(1):30-34.
- (24) Tsai , E.(2012). Endometriosis Basic concepts and current Research trends / Chapter 13:- Stem Cell as the Novel Pathogenesis of Endometriosis. ISBN 978-953-51-0524-4.Hard cover , 490 Pages.
- (25) Rier , S. & Yeaman , G.(2013) . Immune Aspects of Endometriosis: Relevance of the Uterine Mucosal Immune System. Semin Reprod Med, 15(3): 209-220.
- (26) Giudice , L. & Kao , L.(2004). Endometriosis. Lancet ,13-19;364(9447):1789-99.
- (27) Uwaezuoke, S., Udoye,E. & Etebu, E.(2013). Endometriosis of the appendix presenting as acute appendices: A case report and literature review. Ethiop J Health Sci, 23(1):72-69.
- (28) Khatua,B. , Roy, S. & Mandal,C. (2013). Sialic acids siglec interaction: A unique strategy to circumvent innate immune response by pathogens. Indian J. Med. Res, 138, 648-662.
- (29) Chi Chang , Y. , Olson , J. , Beasley ,F. et al. (2014). Group B streptococcus engages a inhibitory siglec through Sialic acid Mimicry to blunt innate immune and inflammatory responses in vivo. J. of DLOS Pathogens , 10(1): e1003846.
- (30) Bradova, A. , Zidkova , J. , Peltre , G. & Ulcova-Gallova,Z.(2012). IgG , IgA and IgE Reactivities to serum Antigens in infertile women. Jorden Journal of biological science ,5(2):85-89.
- (31) Dalziel, M.(2014). Emerging Principles for the Therapeutic Exploration of Glycosylation. J. of Science, 343:DOI:10.1126/1235681.
- (32) Sorial ,S. , Tempest,N. & Hapangma ,D.(2013). Theories on the Pathogenesis of Endometriosis. International Journal of Reproductive Medicine, Article ID 179515, 9 pages.
- (33) Guo, S.(2009). Recurrence of endometriosis and its control. Human Reproduction update. 15(4):441-461.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
<http://www.iiste.org>

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: <http://www.iiste.org/journals/> All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: <http://www.iiste.org/book/>

Recent conferences: <http://www.iiste.org/conference/>

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digital Library , NewJour, Google Scholar

