

Effect of Clomiphene Citrate on Ovulation Induction and Hormones of Infertility with Inflammatory Effects on Gingiva

ASMA ARSHAD¹, SAIMA PERVAIZ², ABEERA ZAFAR³, SARA MASOOD CHEEMA⁴, MARYAM KHAN⁵, FAIZA KHAN⁶, SABA SHAMIM⁷

¹Assistant Professor, Biochemistry Department, Continental Medical College, Lahore.

²Assistant Professor Pathology, Azra Naheed Dental College, Superior University, Lahore.

³MBBS, FCPS (Gynae /obs)

⁴Assistant Professor Pathology, Azra Naheed Medical College, Superior University, Lahore.

⁵Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore.

⁶Associate Professor Pharmacology, Pak Red Crescent Medical and Dental College, Lahore.

⁷Associate Professor, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore.

Correspondence to: Dr. Saima Pervaiz, Email ID: saimapz@hotmail.com

ABSTRACT

Objective: To determine clomiphene citrate effect on ovulation induction and its effects and evaluation on inflammation of gingiva.

Materials and Methods: This was a clinical trial on 50 patients using clomiphene citrate [CC] for ovulation induction and 50 patients with control. These women were examined for their dental hygiene, and to evaluate the index of gingiva, and index of plaque, gingival crevicular fluid, and how much bleeding occurs when probing, any inflammation and dental caries. All the results were compared with a control group in this evaluation [50 females] who do not use ovulation medicines.

Results: Clomiphene citrate is used for ovulation induction. It alters the hormonal level in serum and also leads to gingival inflammation. Although same level of plaque is $P > 0.05$, females having CC treatment for ovulation induction for more than three to four months with increase inflammation pg gingiva level respectively having value of $P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively), GCF volume is $P < 0.001$) and bleeding is with $P < 0.001$ as it was compared with control group and to treatment with CC for three months..

Conclusion: It is concluded that hormonal disturbance in infertility and drugs used in present study may cause gingival inflammation.

Keyword: Clomiphene citrate, luteinizing hormone, follicle stimulating hormone, inflammation, Gingivitis.

INTRODUCTION

A big percentage of couples from all around the world have been facing the problem of Infertility for decades. Unproductiveness is the incapacity of a married couple to accomplish conception after living together for one year, and they are not using any contraceptive method. The main reason for this infertility in females is due to hormonal imbalance resulting in anovulation.¹ Generally, Ovulation induction method used for treatment of infertility, this method is used for the females with anovulation and also for other disorders such as anovulation or amenorrhea but also for those couples who have low sperm count and difficulty to conceive.² Numerous ovulation induction drug systems have been used including Clomiphene citrate (CC), the methods of infertility treatment such as laparoscopic ovarian drilling, and use of medicines aromatase inhibitors, and

Induced the ovulation with hormone Gonadotropins. Medicines have been used to enhance ovulation that results in reduction in serum progesterone and estrogen levels and sends signals to higher centers in hypothalamus that release hormones gonadotropins which help in development of the follicles and induction of the ovulation process. Ovulation is commonly used method for infertility treatment in females having ovulatory problems like amenorrhea and infertility due to anovulation, it used for the treatment when there is any blockage in fallopian tubes in females, defective sperm, low sperm level and unsolved aspects.³ Gonadotropins and Clomiphene citrate (CC) such as follicle stimulating hormone (FSH) and other hormones like and human menopausal gonadotropin are used to treat the infertility due to anovulation.³⁻⁵

The receptors of hormones Estrogen and progesterone receptors are present on Gingiva, any elevation of these hormone levels in puberty⁶ and pregnancy⁷ are linked with periodontal diseases. Generally use of Ovulation Induction drugs results in increased levels of serum progesterone, estrogen and other female sex hormones.⁸ This mechanism is different and usually depends on certain aspects.^{9,10} Fertility hormones in females cause change in the vascular tissue system in gingiva, depress cell-mediated immune system and control subgingival flora.¹¹ This study is conducted to evaluate the effect of clomiphene citrate on gingiva which leads to gingival inflammation when used for

infertility treatment.

Objectives of this study: To reproduce in humans is a very natural and basic instinct and to control fertility there are certain methods. In Pakistan infertility due to PCOS is about 52%.

The study will help to evaluate and demonstrate clomiphene citrate effect for ovulation induction and its effect gingiva which is affected by causing its inflammation.

MATERIALS AND METHODS

The test group comprised females who had 25 teeth present and had been getting treatment and exposed to induce the ovulation. Enrolled participants were divided into a control group and a group which is taking CC, into groups according to drug protocol and drug usage for treatment.

Evaluation for the female hormonal profile. The hormone level is evaluated with ELISA technique. This is based on a noncompetitive sandwich method for infertility hormones. For measurement of hormonal level ELISA kits were used.

Inclusion criteria: Patients diagnosed with infertility and are suffering from gingivitis

Presence of 25 teeth in oral cavity

Exclusive criteria: All patients having OHSS, diabetes, thyroid problem and hyperprolactinemia. Systemic underlying disease

Antibiotics and Anti Inflammatory Drugs usage during the past 3 months. Any periodontal treatment Taken in the past 6 months.

Also exclude those Patients having advanced gingivitis and periodontitis, hyperplasia of gums, acute bleeding gums, gingival ulcers and periodontal abscess.

Ovulatory diseases, e.g asymmetrical Menstrual cycles, polycystic ovary syndrome, hypothalamic or pituitary amenorrhea, or hyperprolactinemia and premature ovarian failure were excluded from study

Study Population: 100 patients were included in this study in which 50 were infertile women taking CC for ovulation induction and having gingival inflammation. Written informed consent was taken from all participants.

Collection of Clinical Data: All enrolled participant's clinical data was collected in their normal cycle at the day 14, to reduce likely menstruation effects on gingiva. Both studied there the control

group was inspected for the inflammation of gingiva and level of plaque and bleeding occurred on probing [12]. Take four sides record from each tooth excluding third molars: mesial and the distal one, buccal also the lingual. Bleeding on probing occurs from these four sites in the time of 30 seconds, the percentage of the bleeding then measured. Manual periodontal probe was used to take measurements by trained instructors for the study groups and for control groups.

Gingival Crevicular Fluid (GCF) Collection: Single rooted teeth selected and got two Gingival Crevicular Fluid testers from every research

Individual. Particularly from distal and mesial interproximal surfaces [13]. Before taking samples, the plaque if attached to teeth surfaces was removed, also isolate the teeth surfaces with cotton balls or air dry them. Carefully place filter paper [Periopaper] into the sulcus deep where minor resistance felt, then keep paper there for 30 seconds. Collected GCF volume was assessed by reference to the standard curve. More the volume of GCF recorded the more will be the severity of Gingivitis and Periodontitis.

Statistical Analysis: SPSS version 23 was used. ANOVA test was applied to rule out the relationship between three groups Control, CC given more than 3 cycles and CC given more than 3 cycles with Periodontal Index, Gingival Index (GI), Gingival Crevicular Fluid and percentage of bleeding gums. Results presented as mean ± standard deviation (SD).

RESULTS

Plaque index was scored as 0-3 which is considered as excellent, 4-7 as good, 8-11 as fair and 12-18 as poor. The differences between mean values of plaque index scores of the study groups and the control group value is P=0.05, signifying that the health of oral hygiene of the groups was related (Table 1).

Gingival Index: Gingival Index which scores each site on 0 to 3 scale, with 0 normal and 3 severe inflammation described inflammation and bleeding, redness and if edema is present. There was a difference between median values of gingival index with value P = 0.000, and when this was compared with the control group, it showed more gingival index score and the difference was significant for CC P=0.01.

Bleeding: Bleeding in these groups was different from each other P = 0.000. The scores were higher in the CC > 3c group when compared to the controls (P < 0.001) and to the CC < 3c group (P = 0.05). (Table 1).

GCF Volume: There was also difference in Three groups for mean values of GCF volume (P = 0.000). The CC >3c and CC >3c had significantly increased GCF volumes in comparison with controls (P < 0.001) (Table 1).

Table 1. Statistical analysis for Plaque Index, Gingival Index Percentage of Bleeding Sites, and Gingival Crevicular Fluid in the Study Population (mean ± SD).

	Control	CC < 3 cycles	CC > 3 cycles
N	20	18	16
PI	0.86 ± 0.43	0.84 ± 0.28	0.87 ± 0.43
GI	1.16 ± 0.45	1.26 ± 0.26	1.57 ± 0.27
Bleeding Sites (%)	10.15 ± 7.05	14.38 ± 7.7	21.56 ± 6.63
GCF volume (ul/2 sites)	0.49 ± 0.17	0.73 ± 0.25	0.93 ± 0.25

DISCUSSION

It has been proved in many studies that changes in sex hormonal levels may effect periodontal tissues. The metabolism of bone is also effected by steroid sex hormones. The cell metabolism and growth in target tissue is known to be influenced by sex steroid

hormones. Levels of estrogen may affect the homeostasis of epithelium, connective tissue, vascularity, and production of keratin and collagen. Definitely, in pregnancy, function of the epithelial cell is likely to decrease and results in reduced degree gingival keratinization. Fibroblast proliferation and collagen maturation was induced by progesterone and Estrogen in connective tissue. Progesterone changes rate and form of collagen production in gingiva that results in collagen turnover change.¹⁰ In target tissues Progesterone increases the absorbance of blood vessels. The resulting stasis of intravascular cellular flow, Hemoconcentration that follows Transendothelial migration and extravascular inflammatory cells are all very similar to influence on degree of gingival erythema, edema and hyperplasia.¹¹ Clomiphene citrate is a drug which is an anti-estrogen and decrease the serum LH and its premature surge.⁶ In a cohort study by Branigan et al It also prevented the hypersecretion of LH. Altered level of FSH, LH affects the normal fertility in females. There is also a decrease in the Progesterone due to decrease in the luteal phase surge. Among these, increased levels of prolactin also cause infertility in females.⁷

Increase serum concentration of progesterone, estrogen and other sex hormones can effect gingival micro vascularization 31 that results in increased gingival crevicular fluid and gingival edema [14]. Colonization of extremely pathogenic bacteria like P. intermedia can be promoted by Estrogen that may also work like a growth factor.¹⁵ Moreover, increased concentration of progesterone and estrogen can change the host defense process in gingiva and decrease neutrophil chemotaxis and phagocytosis.¹⁶ Although in case of CC it usually binds with receptor E2 in many tissues like uterus, ovaries, cervix, hypophysis and hypothalamus.¹⁷ For further studies evaluating binding of CC and estrogen receptors of gingiva and concentration of CC.

CONCLUSION

Conclusion of study shows that medicines are commonly used for infertility for induction of ovulation and clomiphene citrate is one of them used as a primary drug. For this purpose. The main diseases of periodontics include bleeding gums. There should be further studies to evaluate the effect if medicine affects the gingiva in infertility treatment.

REFERENCES

1. Ainavi, I.I., Pattern of Reproductive Hormones in Women with Infertility in Zaria, Northern Nigeria (Doctoral dissertation). 2009: p. 1-70.
2. Xu, X.L. and S.L. Deng, Estrogen Receptors in Polycystic Ovary Syndrome. 2021. **10**(2).
3. Teede, H., A. Deeks, and L. Moran, Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med, 2010. **8**: p. 41.
4. Shi, S., et al., Letrozole and human menopausal gonadotropin for ovulation induction in clomiphene resistance polycystic ovary syndrome patients: A randomized controlled study. Medicine (Baltimore), 2020. **99**(4): p. e18383.
5. Rashidi, M., et al., Advantages of recombinant follicle-stimulating hormone over human menopausal gonadotropin for ovarian stimulation in intrauterine insemination: a randomized clinical trial in unexplained infertility. Eur J Obstet Gynecol Reprod Biol, 2013. **169**(2): p. 244-7.
6. Oh, T.J., R. Eber, and H.L. Wang, Periodontal diseases in the child and adolescent. J Clin Periodontol, 2002. **29**(5): p. 400-10.
7. Yalcin, F., et al., The effect of sociocultural status on periodontal conditions in pregnancy. J Periodontol, 2002. **73**(2): p. 178-82.
8. Parkar, M.H., H.N. Newman, and I. Olsen, Polymerase chain reaction analysis of oestrogen and androgen receptor expression in human gingival and periodontal tissue. Arch Oral Biol, 1996. **41**(10): p. 979-83.
9. Mariotti, A., Sex steroid hormones and cell dynamics in the periodontium. Crit Rev Oral Biol Med, 1994. **5**(1): p. 27-53.
10. Mealey, B.L. and A.J. Moritz, Hormonal influences: effects of diabetes mellitus and endogenous female sex steroid hormones on the periodontium. Periodontol 2000, 2003. **32**: p. 59-81. 11. Wu, M., S.W.

- Chen, and S.Y. Jiang, Relationship between gingival inflammation and pregnancy. *Mediators Inflamm*, 2015. **2015**: p. 623427.
12. Demmer, R.T., D.R. Jacobs, Jr., and M. Desvarieux, Periodontal disease and incident type 2 diabetes: results from the First National Health and Nutrition Examination Survey and its epidemiologic follow-up study. *Diabetes Care*, 2008. **31**(7): p. 1373-9.
 13. Buduneli, N., et al., Gingival crevicular fluid matrix metalloproteinase-8 levels following adjunctive use of meloxicam and initial phase of periodontal therapy. *J Periodontol*, 2002. **73**(1): p. 103-9.
 14. Kashetty, M., et al., Oral hygiene status, gingival status, periodontal status, and treatment needs among pregnant and nonpregnant women: A comparative study. *J Indian Soc Periodontol*, 2018. **22**(2): p. 164-170.
 15. Aruni, A.W., et al., The Biofilm Community-Rebels with a Cause. *Curr Oral Health Rep*, 2015. **2**(1): p. 48-56.
 16. Knight, E.T., et al., Risk factors that may modify the innate and adaptive immune responses in periodontal diseases. *Periodontol* 2000, 2016. **71**(1): p. 22-51.
 17. Paulose, T., et al., Estrogens in the wrong place at the wrong time: Fetal BPA exposure and mammary cancer. *Reprod Toxicol*, 2015. **54**: p. 58-65.