e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2022; 12(4); 159-166

Original Research Article

Prospective, Randomized Comparative Assessment of Efficacy of Chloroquine Phosphate 0.03% And Sodium Carboxymethylcellulose 1% in Dry Eye

Kundan Kumar Singh

Senior Resident, Department of Opthalmology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India

Received: 14-02-2022 / Revised: 15-03-2022 / Accepted: 10-04-2022
Corresponding author: Dr. Kundan Kumar Singh
Conflict of interest: Nil

Abstract

Aim: A comparative study of efficacy of chloroquine phosphate 0.03% and sodium carboxymethylcellulose 1% in dry eye

Methods: This prospective, randomized, study was done in the department of Opthalmology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India for 2 yeas. The protocol was composed of 2 phases: a 3-week treatment phase, and a 1-week post treatment phase. Eligible patients were between 20 to 68 years of age.

Results: The group mean scores was found to be (2.89±0.12) at baseline and (0.32±0.04) after treatment (P< 0.001) with the net change -2.67 (95% CI of -2.93 to -2.42). It is also to be noted that CHQ also showed significant reduction in LGSS even at visit 1 & 2 (P<0.001). CMC treatment showed significant change in mean score only at visit 3 i.e. 1.21±0.11 (V3) from 1.88±0.13 (BL) with a net change of -0.97 (95% CI of - 1.31 to -1.65) (P<0.05) CHQ treated group reflected significant reduction in FLSS from 3.31±0.13 (BL) to 0.57±0.07 (V3) (p<0.001) with the net change of -2.84 (95% CI of -3.017 to -2.551). Significant reduction was noted at the end of visit 1 (Table 2). CMC treated group indicated significant reduction in FLSS only at visit 3 from 1.62±0.01 as compared to 2.81±0.14 at baseline (P <0.05) with a net change of just -0.49 (95% CI of -1.82 to -1.16). Further, the percentage improvement in CHQ and CMC groups were 50% and 29% respectively.

Conclusion: The findings of this study support the continued investigation of the use of topical CHQ as a safe and effective treatment for DES. In conclusion, Chloroquine Phosphate eye drops can be a novel therapeutic approach for the restoration of tear formation for DES.

Keywords: DES, chloroquine phosphate, sodium carboxymethylcellulose

Introduction

DED is a chronic pain syndrome affecting quality of life adversely in patients and even affecting outcome of surgeries for cataract, refractive errors and glaucoma. [1] It is characterized by instability of the tear film, with or without inflammation of the ocular surface and lacrimal glands [2] and accompanied by increased osmolality of tear

film and inflammation of the ocular surface.v [2-4] The dry eye workshop study classifies dry eye as: [3] Aqueous deficient dry eye i.e. failure of lacrimal tear secretion as seen in Sjogren's syndrome, lacrimal gland deficiency, lacrimal duct obstruction, reflex blockage or as a result of systemic drug toxicity. Evaporative dry eye due to excessive evaporation of the aqueous component secondary to deficient lipid component as seen in meibomian gland dysfunction, lid disorders, contact lens wearers, vitamin A deficiency and topical drug toxicity.

Patients usually present to the ophthalmologist with complaints of foreign body sensation, ocular dryness, ocular grittiness, hyperemia, ocular irritation, burning, itching, photophobia and fluctuation or blurring of vision associated with redness of eyelids and conjunctiva. Ocular examination may reveal stringy mucus and particulate matter in the tear film. Ocular surface becomes lusterless. There is conjunctival xerosis or Bitot's spots in the conjunctiva and filamentary keratitis may be present. [5] Various studies have revealed the inflammatory component to be the main causative factor of the disorder Cytokine and receptor mediated inflammatory cascade6 and apoptosis have also been implicated in the pathogenesis of dry eyes. [7] With a better understanding of the underlying pathogenesis, goal for treatment of patients with DED has shifted from merely improving the patient's ocular comfort and quality of life to return of the ocular surface and tear film to the normal homeostatic state. Current therapies for the management of dry eye include drugs for tear supplementation, retention and stimulation; anti-inflammatory agents, and environmental strategies. [6] Newer techniques include lipiflow [8,9] and punctum occluders. [10] Palliative therapies like tear substitutes are currently the most common choice of treatment but have failed to yield high success rates because they give only symptomatic improvement and do not correct underlying cause. [6]

Material and methods

This prospective, randomized, study was done in the department of Opthalmology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India for 2 years

The protocol was composed of 2 phases: a 3-week treatment phase, and a 1-week post treatment phase. Eligible patients were between 20 to 68 years of age.

Methodology

One or more moderate dry eye–related symptoms, including itching, burning, blurred vision, foreign body sensation, dryness, photophobia, and soreness or pain i.e. a Ocular Surface Disease Index [OSDI] score between 13 and 100,best visual acuity of 6/18 or better in each eye. Patients were excluded from study if they had any ocular disorder including ocular injury, infection, non-dry eye ocular inflammation, trauma, or surgery within the prior 6 months; any were receiving concurrent treatment that could interfere with interpretation of the study results; had any uncontrolled systemic disease or significant illness; contact lens wearer, subject that require surgical correction of dry eyes, known hypersensitivity to chloroquine, chronic alcoholics, pregnant, willing to get pregnant or nursing women or patient with Sjogren syndrome. A total 200 eyes of 100 patients with bilateral dry eyes were recruited. The assignment of patients was based on simple computer-based randomization by the study statistician before the initiation of the study. An equal probability randomization procedure was used. All the patients were randomly assigned in to 2 different treatment groups i.e. Group: 1 Carboxymethyl Cellulose (CMC) (Dose: 0.5 %, 2-4 times / day), Group: 2 Chloroquine phosphate (CHQ), (Dose: 0.03%, twice a day).

All the subjects received treatment for 21 days during which they were evaluated on visit 1 (day 0), visit 2 (day 7), visit 3 (day 14) and visit 4(day 21). Seven days after termination of the treatment subjects were assessed on visit 5(day 28). After taking complete history, patients were examined in accordance with internationally accepted measurement systems i.e. Lissamine green staining score (LGSS), Fluorescein staining of cornea (FLSS), Schirmers test (without anesthesia), symptoms related ocular examination (OSDI) and safety measurements at the commencement of therapy and at every week thereafter. Assessment of tear film and epithelial integrity was carried out by fluorescein and lissamine green dyes used to view any conjunctival and corneal epithelial abnormalities.

Lissamine green staining was performed in both eyes using impregnated lissamine green strips wetted with non preserved, balanced saline solution with results observed in the low- to moderate intensity white light of the slit lamp after 1 minute. Similarly, fluorescein was applied to the eye. Interpretation of ocular surface staining by lissamine green dye was done by van Bijsterveld grading scale [15] and with Fluorescein dye was done by Baylor's score [1] to quantify the intensity of staining. Aqueous secretion was measured by schirmer test without anesthesia.[16] Patient response to treatment was evaluated using the OSDI, a global assessment parameter consisting of 12 questions designed to assess the symptoms of ocular irritation consistent with dry eye disease and their impact on vision-related functioning. The questions covered three areas: ocular symptoms, environmental triggers, and vision-related function. Each question was phrased in terms of frequency and graded on a numerical scale from 0 to 4 by an OSDI score: 0= none of the time; 1= some of the time; 2= half of the time; 3= most of the time; 4= all of the time. Patient responses to all answers were then combined for a composite OSDI score ranging from 0 to 100.[17]

Results

A total of 29 (29 %) male and 71(71%) female patients were reflecting mean age of 54 ± 9.5 years. There were no statistically significant differences in age, gender, and pretreatment tear film and ocular surface parameters between the two groups The efficacy measures were in terms of LGSS, FLSS, Schirmer test and the global assessment scoring system - OSDI. Based on our data, group mean values, as well as the magnitude of the net change in LGSS for any particular cohort were calculated. In addition, relative changes in LGSS were calculated as the percentage change from baseline. CHQ treated group showed significant reduction in LGSS at all the visits ($P<0.05$). The group mean scores was found to be (2.89±0.12) at baseline and (0.32±0.04) after treatment ($P< 0.001$) with the net change -2.67 (95% CI of -2.93 to -2.42). It is also to be noted that CHQ also showed significant reduction in LGSS even at visit 1 & 2 ($P<0.001$). CMC treatment showed significant change in mean score only at visit 3 i.e. 1.21±0.11 (V3) from 1.88±0.13 (BL) with a net change of -0.97 (95% CI of -1.31 to -1.65) ($P<0.05$) (Table 1).

The above analysis indicated some differences in the treatment efficacy of two treatment groups. The next step was to make more specific comparisons to see how robust (or otherwise) any such apparent differences in efficacy might be. Treatment with CHQ showed substantial changes in terms of significant reduction in LGSS as compared to CMC. Analysis of comparison of both the treatments vs. baseline scores on a percentage basis was 92% and 44% respectively for CHQ and CMC treatments. CHQ treated group reflected significant reduction in FLSS from 3.31±0.13 (BL) to 0.57±0.07 (V3) ($p<0.001$) with the net change of -2.84 (95% CI of -3.017 to -2.551). Significant reduction was noted at the end of visit 1 (Table 2). CMC

treated group indicated significant reduction in FLSS only at visit 3 from 1.62±0.01 as compared to 2.81±0.14 at baseline (P <0.05) with a net change of just -0.49 (95% CI of -1.82 to -1.16). Further, the percentage improvement in CHQ and CMC groups were 50% and 29% respectively.

Baseline values for schirmer tear strip wetting scores ranged from 12.53 to 12.88 in both the treatment groups. The most consistent improvement was observed in the CHQ treated group, with mean increase in wetting length of (13.49±0.41), (14.24±0.39), (14.85±0.39) and (14.88 ±0.38) mm at week one, two, three and four respectively when compared with the baseline (12.53±0.44) values. These increases approached statistical significance at week 2 (P<0.05); week 3 (P <0.001) and week 4 (P<0.001) (table 1). The significant improvement from baseline occurred in the CMC group at treatment week 3 only (P<0.05). Further, in support of above findings, a net change of 2.62 (95% CI of 1.47 to 3.77) in mean schirmer value of CHQ treated patients was observed as compared to a net change of 1.24 (95% CI of 0.46 to 1.82) with CMC group. It is to be noted that the % improvement was found higher with CHQ treatment (21%) as compared to CMC treatment (10%). Baseline OSDI scores ranged from 55 to 61 (on a scale from 0 to 100, where 0 indicates no disability and 100 indicate complete disability) in both the treatment groups. CHQ treated group reflected highly significant reduction in OSDI at visit 3 (18.81±1.5) as compared to baseline (61.52 ± 2.21) with a net change of -42.81 (95% CI of -47.5284 to -39.9) (p<0.05) (Table 1).

The mean baseline score for CMC treated group changed significantly (p<0.05) from 54.63±1.43 (BL) to 32.39±1.26 (V3) with a net change - 22.34 (95% CI of -25.79 to -18.89). Both CHQ and CMC treated groups indicated significant reduction in OSDI at every visit when compared with the baseline. Thus, substantial change in OSDI has been achieved with the use of CHQ in terms of net reduction of score. Based upon these findings, we further tried to segregate different categories of problems i.e. ocular symptoms, vision related functions and environmental triggers in patients receiving CHQ. As shown in, mean OSDI significantly decreased from baseline to final assessment (V3) in all 3 categories of problems indicating robust improvement with CHQ treatment. Percentage improvement in CMC and CHQ treated groups were found 41% and 70% respectively.

Table 1: Changes of efficacy measures with different treatment groups

Efficacy Measures	Baseline value(BL) (Day 0)	After treatment				P Value
		V1 (Day 7)	V2 (Day 14)	V3 (Day 21)	V4 (Day 28)	
Lissamine Green Stain Score (LGSS)						
CMC	1.88±0.13	1.81±0.31	1.34±0.2	1.21±0.11*	1.12□0.12*	P<0.05
CHQ	2.89 ±0.12	1.84 ±0.10*	0.82±0.07*	0.32 ± 0.04*	0.24 ±0.03*	P<0.001
Fluorescein Stain Score (FLSS)						
CMC	2.81±0.14	2.54 ±0.13	1.82±0.12	1.62±0.01*	1.46±0.09*	P<0.05
CHQ	3.31±0.12	1.89±0.10*	1.12±0.09*	0.57±0.07*	0.50±0.06*	P<0.001
Schirmer test						

CMC	12.88±0.3	13.34±0.3	13.87□0.2	13.82±0.2*	13.75□0.26	P<0.05
CHQ	12.53±0.4	13.49±0.4	14.24±0.3*	14.85±0.3*	14.88±0.38*	P<0.001
Ocular Surface Disease Index (OSDI)						
CMC	54.63±1.3	45.36±1.1*	37.82±1.2*	32.39±1.1*	33.62±1.8*	P<0.05
CHQ	61.52±2.1	43.1±1.9*	28.46±1.8*	18.81±1.5*	16.77±1.4*	P<0.05

Anova followed by Tukey's test. Asterisk indicates significant difference from baseline (p < 0.05).

Table 2: Treatment Related Adverse Events

Adverse Event	CMC (n=50)	CHQ (n=50)	Total Events (n=100)
Conjunctival hyperemia	1	1	2
Burning eye	1	2	3
Pain in the eye	0	1	1
Visual disturbance	1	0	1
Total patients	3	4	7

Discussion

Occurrence of damage to ocular surface, mainly cornea and conjunctiva are the major signs observed in DES. The extent of damage to conjunctiva can be easily evaluated by LGSS, and to the cornea with the help of FLSS. So, during the treatment of DES, improvement in both the staining score is expected. Similarly, improved secretion of lacrimal gland is desirable with the improvement in disease condition which can be measured by schirmer test.

Comparing both the therapies tested, CHQ treatment produced the most consistent improvement in objective (LGSS, FLSS and Schirmer test) as well as subjective end points (OSDI). The results of the current investigation carried out on a representative sample of mild to severe dry eye sufferers, demonstrated a superior performance for CHQ than for CMC in controlling conjunctival anomalies. The ocular parameter of interest in the present study was conjunctival staining (LGSS). The presence of such staining is indicative of ocular surface desiccation leading to symptoms;[11,12] the relief of staining in that area indicates that the ocular surface has returned to a normal status. Conjunctival staining indicates epithelial cell damage and therefore, necessarily damage to the overlying gel-like mucin layer. There is incomplete coverage of the surface by an unbroken tear film at all times between blinks leading to incomplete surface lubrication, in the exposed area. [13] The greater efficacy of CHQ than CMC can be hypothesized to be linked to anti-inflammatory effect of CHQ as compared to symptomatic relief by lubricating effect of artificial tears. Corneal fluorescein staining was improved significantly after CHQ treatment as early as the first week. Decreasing corneal fluorescein staining is due to the suppression of inflammation enabling normal function of ocular surface. With the deterioration of dry eye, there can be some filament and piece staining in the cornea. It has been confirmed in many clinical and elementary experiments that the inflammatory factors and the marks concerned are diminished after anti- inflammatory treatment. [14] We also found that some patient's vision was improved when the inflammation of ocular surface was relieved.

In the support of above, slow elongation of Schirmer value was observed after the application of CHQ and significantly good effect was observed 21 days after treatment. This can be explained by decreased inflammatory factors and improved integrity of ocular surface after the application of CHQ**,** so the nerves of the cornea and conjunctiva can be stimulated more effectively by blinking, the reflective secretion becoming normal, then the quality and quantity of tears might get improved, which supports the improved results of lacrimal gland secretion by Schirmers test in our experiment. Further, CHQ treated group showed significant relief from symptoms for different categories namely ocular symptoms, vision related functions and environmental triggers based on the results of % OSDI score.

The lysosomotropic effects of CHQ are widely believed to be responsible for its anti-inflammatory properties and effectiveness in the treatment of some autoimmune diseases. [15] It is reported that CHQ decreases the production of the pro-inflammatory cytokines IFN-alpha, tumour necrosis factor-alpha (TNF-alpha), and interleukin-6 (IL-6) in Lipopolysacharide (LPS)-or phytohemagglu-tinin stimulated peripheral blood mononuclear cells [16], and also augmented LPS-induced expression of TNF-alpha and IL-6 in monocytic and microglial cells. [17] CHQ also known to exert anti- inflammatory effects via non-lysosomotropic mechanisms. It is shown to inhibit TNF-α release in macrophages through inhibition of TNF-α mRNA synthesis, thereby suggesting that it can also disrupt gene transcription [18-20] but does so without interfering with posttranslational modification or release of the cytokine from macrophages. [21] In human histolytic U-937 cells, CHQ has shown to decrease cell surface expression of TNF- α receptors by retarding their transport to the cell surface.[22] The blocking of pro-inflammatory cytokines by CHQ was shown to be protective against LPS- and Escherichia coli DNA-induced inflammatory responses and/or sepsis in mice. [23] CHQ also inhibits cytokine release into human whole blood, an effect that could be beneficial in diseases that are related to bacterial-induced inflammation. [24] CHQ inhibits metalloproteases liberated by macrophages, neutrophils and the dead or dying cells. Irrespective of some controversies regarding minute details of mechanism of action, the drug has been widely used in arthritis for decades. Topical CHQ administration may offer similar advantages in dry eyes. It is already reported that the physiological, cellular, and biochemical effects of CHQ are exerted through pleiotropic mechanisms involving both lysosomotropic-dependent and independent effects. This cornucopia of mechanisms of action has seen CHQ persist on therapeutic regimens for several diseases and conditions despite its systemic toxicity and the emergence of drug resistance in malaria parasites. [25] In any ocular insult and inflammation, there is an increased epithelial turnover that exposes immature cells to UV radiations, visible light or other environmental factors. Topical CHQ is reported as protective against UV radiations, particularly UVB and UVA induced erythema in skin.[26] Besides anti-inflammatory properties, CHQ could also have photo-protective effects in conditions such as lupus erythematosus 34 and could be exploited in the dry eye disease via protecting localized cell mediated inflammatory processes which contribute to the development of it. Toxicity and adverse effects of CHQ are well documented in the literature. But they are related to high cumulative systemic dose. CHQ when given topically at 0.03% dose twice a day for 21 days, as in present study, the total dose reaching local ocular tissue or absorbed systemically is very minute fraction of the toxic cumulative dose. Thus in particular, the superiority of CHQ was observed in both a primary objective endpoint (lissamine green staining scores) and a

primary subjective endpoint (global symptom frequency scores) in the same study. The trend observed in certain secondary objective and subjective efficacy endpoints at Day 7 and/or Day 14 demonstrated the beneficial effects of the drug at and beyond the initial (7- day) endpoint observation, providing additional reinforcement to the findings in the primary endpoints. So there is no question of any local or systemic side effect specific for CHQ. In the present study, the most important safety findings were in terms of documenting adverse effects. [27] The safety of CHQ is well established in our study and the benefit-to-risk evaluation is overwhelmingly positive. The importance of this study in providing the scientific evidence supporting the efficacy of CHQ in the treatment of the signs and symptoms of dry eye disease is considerable. CHQ, despite its well documented toxicity and adverse effects may have important future uses that are associated with its lysosomotropic and immunomodulatory mechanisms.[26]

Conclusion

The findings of this study support the continued investigation of the use of topical CHQ as a safe and effective treatment for DES. In conclusion, Chloroquine Phosphate eye drops can be a novel therapeutic approach for the restoration of tear formation for DES.

References

1. Roy NS, Wei Y, Kuklinski E, Asbell PA. The Growing Need for Validated Biomarkers and Endpoints for Dry Eye Clinical Research. Investig Opthalmol Vis Sci. 2017;58(6):BIO1.
2. Javadi MA, Feizi S. Dry eye syndrome. J Ophthalmic Vis Res. 2011;6(3):192–8.
3. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop. Ocul Surf. 2007;5(2):75–92.
4. Maurya RP. Dry eye disease: An overview. Indian J Clin Exp Ophthalmol. 2018;4(4):433–4.
5. Mishra B. Prevalence of Dry Eye and Its Association with Various Risk Factors in Rural Setup of Western Uttar Pradesh in a Tertiary Care Hospital. Open J Prev Med. 2016;6(10):4236.
6. Pflugfelder SC. Antiinflammatory therapy for dry eye. Am J Ophthalmol. 2004;137(2):337–42.
7. Gao J, Schwalb TA, Addeo JV, Ghosn CR, Stern ME. The Role of Apoptosis in the Pathogenesis of Canine Keratoconjunctivitis Sicca. Cornea. 1998;17(6):654–63.
8. Greiner JV. A single LipiFlow Thermal Pulsation System treatment improves meibomian gland function and reduces dry eye symptoms for 9 months. Curr Eye Res. 2012;37(4):272–8.
9. Dorennavar L, Maurya RP, Singh VP, Singh MK, Sharma K, Sharma R. The role of Rebamipide ophthalmic suspension in management of dry eye disease. Indian J Clin Exp Ophthalmol. 2015;1(4):191–6.
10. Ohba E, Dogru M, Hosaka E, Yamazaki A, Asaga R, Tatematsu Y, et al. Surgical Punctal Occlusion With a High Heat-Energy Releasing Cautery Device for Severe Dry Eye With Recurrent Punctal Plug Extrusion. Am J Ophthalmol. 2011;151(3):483–7.
11. Guillon M, Maissa C. Bulbar conjunctival staining in contact lens wearers and non lens wearers and its association with symptomatology. Cont Lens Anterior Eye 2005;28(2):67–73.
12. Lakkis C, Brennan NA. Bulbar conjunctival fluorescein staining in hydrogel contact lens wearers. CLAO J 1996;22(3):189–94.
13. M. Guillon a, C. Maissa a, S. Hob. Evaluation of the effects on conjunctival tissues of Optive eye drops over one month usage. Contact Lens & Anterior Eye 2010;33:93–99.

14. Avunduk AM, Avunduk MC, Varnell ED, Kaufman HE. The comparison of efficacies of topical corticosteroids and nonsteroidal anti-inflammatory drops on dry eye patients: a clinical and immunocytochemical study. Am J Ophthalmol 2003;136:593-602.
15. Wozniacka A, Lesiak A, Narbutt J, Kobos J, Pavel S, Sysa-Jedrzejowska A. Chloroquine treatment reduces the number of cutaneous HLA-DR+ and CD1a+ cells in patients with systemic lupus erythematosus. Lupus 2007;16:89-94.
16. Van den Borne BE, Dijkmans BA, de Rooij HH, le Cessie S, Verweij CL. Chloroquine and hydroxychloroquine equally affect tumor necrosis factor-alpha, interleukin 6, and interferon-gamma production by peripheral blood mononuclear cells. J Rheumatol 1997;24:55-60.
17. Park J, Kwon D, Choi C, Oh JW, Benveniste EN. Chloroquine induces activation of nuclear factor-kappaB and subsequent expression of pro-inflammatory cytokines by human astroglial cells. J Neurochem 2003;84:1266-74.
18. Weber SM, Levitz SM. Chloroquine interferes with lipopolysaccharide-induced TNF-alpha gene expression by a nonlysosomotropic mechanism. J Immunol 2000;165:1534-40.
19. Zhu X, Ertel W, Ayala A, Morrison MH, Perrin MM, Chaudry IH. Chloroquine inhibits macrophage tumor necrosis factor-alpha mRNA transcription. Immunology 1993;80:122-6.
20. Weber SM, Chen JM, Levitz SM. Inhibition of mitogen activated protein kinase signalling by chloroquine. J Immunol 2002;168:5303-9.
21. Jang CH, Choi JH, Byun MS, Jue DM. Chloroquine inhibits production of TNF-alpha, IL-1beta and IL-6 from lipopolysaccharide-stimulated human monocytes/ macrophages by different modes. Rheumatology 2006;45:703- 10.
22. Jeong JY, Choi JW, Jeon KI, Jue DM. Chloroquine decreases cell-surface expression of tumour necrosis factor receptors in human histiocytic U-937 cells. Immunology 2002;105:83-91.
23. Hong Z, Jiang Z, Liangxi W, Guofu D, Ping L, Yongling L, et al. Chloroquine protects mice from challenge with CpG ODN and LPS by decreasing proinflammatory cytokine release. Int Immunopharmacol 2004;4:223-34.
24. Karres I, Kremer JP, Dietl I, Steckholzer U, Jochum M, Ertel W. Chloroquine inhibits proinflammatory cytokine release into human whole blood. Am J Physiol 1998;274:R1058- 64.
25. Cooper RG, Magwere T. Chloroquine: Novel uses & manifestations. Indian J med Res 2008; 127:305-16.
26. Forsberg GS, Lindström B, Berne B. Topical chloroquine applied before irradiation protects against ultraviolet B (UVB)- and UVA-induced erythema but not against immediate pigment darkening. Photodermatol Photoimmunol Photomed. 1993;9(5):220-4.
27. Alhamdani, F., & Abdulla, E. H. Influence of Patient's Age and Gender on Dental Implant Treatment Five Year retrospective study. Journal of Medical Research and Health Sciences, 2021:4(9), 1461–1467.