Original Research Article

Microfilaricidal Actions of Chloroquine and Doxycycline on *Onchocerca volvulus in vivo*

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Abstract

Background: The study examined the microfilaricidal effects of oral administration of chloroquine and doxycycline on *Onchocerca volvulus* parasites.
Methods: One (1) mg of bloodless skin snip was extirpated from the sterilized posterior iliac crest region of each parasitized participant using a corneo-scleral punch. The skin snip biopsy was immersed in a micro titre plate containing 0.2 ml of normal saline, vigorously agitated and left at the ambient room temperature for 24 hours. The aliquots were observed for emerging microfilariae (mf) which were visualized and counted under a 10X objective microscope prior to drug administration. One hundred (100) participants who tested positive to *O. volvulus* were recruited into the study on consecutive basis and stratified into 2 treatment and 2 placebo clusters. Group A (n=25) was treated with the standard dose of chloroquine and re-quantitated for parasitemia on day 7, while Group B (n=25) was treated with 200mg daily dose of doxycycline for 6 weeks and re-evaluated for parasite load on weekly basis for six weeks. Placebo participants (Control A n=25; Control B n=25) were treated with standard doses of multivitamin for 1 week and 6 weeks respectively.
Results: Chloroquine and doxycycline exhibited clearance rates of 100% on day 7 and week 5-6 respectively. In Groups A and B, the post-treatment Mean Parasite Densities (MPDs) were significantly lower ($p<0.05$) than the pre-treatment levels.
Conclusions: The findings of the present study affirm those of previous studies which suggest that doxycycline could provide a suitable alternative to ivermectin in the treatment of onchocerciasis in Loasis co-endemic areas.
Keywords: Microfilaricide; Chloroquine; Doxycycline; *Onchocerca volvulus*; *in vivo*

Introduction

Human onchocerciasis or river blindness is a chronic parasitic disease caused by the filarial worm known as *Onchocerca volvulus.* Onchocerciasis has been associated with blindness, visual impairments, systemic dysfunctions, lifelong human suffering and grave socioeconomic problems in many endemic communities. Blindness and/or visual impairments are the most serious and overt clinical manifestations of onchocerciasis and are most prevalent among villages around riverine areas [1]. In many endemic communities, the ocular manifestations have been treated with crude and unorthodox procedures resulting in increased burden of blindness due to delayed intervention and severe ocular complications [1].

Onchocerca volvulus is a nematode that belongs to the family *filaridae and* the only onchocerca specie with a human host, although an infected Spider-Monkey and Gorilla have been recorded [2]. The adult worms of *O. volvulus* live mainly in subcutaneous nodules, where the female worm gives birth to millions of microscopic embryos called microfilariae. In Nigeria and other West African countries, the microfilariae are predominantly found in the lymphatic channels of the skin around the pelvic region and upper arm [1]. Microfilariae are transmitted from the infected to the healthy host through the bite of a female black fly which ingests several microfilariae from the infected host and transmits them in the form of infective larvae to the healthy host during a blood meal.

Black flies are tiny ferocious biters and the genus *Simulium* is the only vector of *O. volvulus.* The vector; *Simulium damnosium* breeds in well oxygenated and nourished fast flowing rivers which limits the flies to villages around the river banks [1]. Their eggs require fast flowing rivers for breeding grounds [3] and the adult flies emerge after 8-12 days following egg production; with the ability to travel hundreds of kilometers in flight on wind currents, although there is a strong argument that they can only travel within a radius of 15km and their life span is about four weeks [1].

Doxycycline is a lipophilic bacteriostatic antibiotic and a translation inhibitor that specifically acts by the depletion of Wolbachia endobacteria, inhibition of embryogenesis in female worms and filaricidal activities [4,5] . Studies [4,5] have shown that the administration of 100mg daily dose of doxycycline for 6 weeks resulted in progressive depletion of

wolbachia from adult worms and microfilariae over a period of 6 months, inhibition of embryogenesis after 6 months with respect to all embryo stages, followed by decline in microfilariae after 11 months, reduction in spermatozoa in the female genital tract, depletion/marked reduction in endobacteria and inhibition of embryogenesis; whereas spermatogenesis was only partly reduced after 11 and 18 months.

Chloroquine phosphate has shown reasonable activity against various filarial species. It was reported to have effected 100% reduction in dermal *O. volvulus* microfilariae 7 days after treatment, with no effect on the adult *O. volvulus* examined histologically in extirpated nodules[6] following an *in vivo* treatment. In addition, some studies [7,8] have reported significant morphological changes in nodules infiltrated with chloroquine compared with those infiltrated with saline and those not infiltrated. They argued that chloroquine exhibited macrofilaricidal effects on adult *Onchocerca volvulus* by local infiltration of palpable onchocercal nodules, as well as low motility indices and complete degradation of the internal structures, such as the uterus and intestine of the worms in nodules infiltrated with chloroquine.

Onchocerciasis control is currently based on mass distribution of ivermectin which elicits Severe Adverse Effects (SAEs) in persons co-infected with very high loads of *Loa loa* microfilaria (≥730,000 mf/ml) [9]. SAEs have been reported in ecological zones with significant preponderance of onchocerciasis and loasis co-endemicity during mass distribution of ivermectin [9]. Furthermore, the SAEs elicited by ivermectin during onchocerciasis control in areas of *Loa loa* co-endemicity and the challenges of sustaining the mass distribution of ivermectin by governments and intervention agencies are increasingly becoming worrisome. A useful means of preventing SAEs would be to treat at-risk populations with either a drug that will reduce the *Loa loa* microfilarial load prior to the administration of ivermectin or one that is non-toxic to it.

Although previous studies suggest that chloroquine [9] and doxycycline [5,8,10] generate reasonable activities against various filarial species *in vivo* and *in vitro*, Abegunde *et al.* [11] have opined that the effect of doxycycline on onchocerciasis is unclear, while other studies [12-14] have argued that chloroquine resulted in a marked decrease in *Onchocerca*

volvulus microfilarial loads, and had a macrofilaricidal effect when injected into the onchocercal nodules, but not when administered orally. Therefore, the present study examined the microfilaricidal actions of chloroquine and doxycycline on *O. volvulus in vivo* in order to provide empirical data to guide clinical decisions on the substitution of ivermectin with either chloroquine or doxycycline in the treatment of onchocerciasis in *Loa loa* co-morbidity.

Methods

Study locale:

The study was conducted in Ahani-Achi Community, Enugu State, Nigeria; an autonomous community with geographical coordinates of 6.1221°N 7.3609°E. Climatically, the area is marked with typical rain forest mosaic vegetation characterized by mainly rainy seasons. The community is traversed by many streams and fast flowing rivers such as Iyibenze, Nwoka, Ogba, Iyi Owerri, Iyi Agwo and Ngene iyi agu drained by a principal tributary known as Oji River. It has an annual rainfall of approximately 2,900-3,400 mm, with maximum precipitation occurring from June to August. The main occupations of the people are farming, fishing and trading.

Ethical considerations:

The experimental study was designed in accordance with the 1967 Helsinki Declaration on Human Experiments (as modified in Fortaleza, 2013). The study was approved by the Ethical Committee of the Faculty of Science, Imo State University Owerri, Nigeria and written informed consents of the participants were obtained. The methodology of the study was devoid of all forms of harm and the right of participants to withdraw from the study at any point was communicated in their native language.

Procedure for data collection:

Skin snip biopsy technique was adopted for parasite quantitation; 1 mg of bloodless skin snip was extirpated from the sterilized posterior iliac crest region of each participant using a corneo-scleral punch. The skin snip biopsy was immersed in a small tube containing 0.2 ml of normal saline, vigorously agitated and left at the ambient room temperature for 24 hours. The aliquots were examined under a 10X objective microscope for parasite density. One hundred (100), out of 328 participants tested positive to *O. volvulus* parasites and were recruited into the study.

Treatment allocation was based on randomization. The parasitized participants

were stratified into two groups; Group A was comprised of 25 participants treated with the standard dose of chloroquine phosphate (1000mg starting dose, followed by 500mg after 6-8 hours, then 500mg each day for 2 days), and 25 controls on a placebo standard dose of multivitamin tablets for one week, while Group B had 25 participants treated with 200mg daily dose of doxycycline for six weeks and 25 controls placed on a placebo standard dose of multivitamin for six weeks. The post-treatment parasite densities of both groups were re-examined using the same technique. In group A, the post-intervention parasite load was enumerated on day 7, while in group B, the post-treatment parasite load was counted on weekly basis for six weeks.

Data analysis

Data were analyzed using descriptive (percentages) and inferential (Chi-square (X^2)) statistics. $P<0.05$ was considered statistically significant at 95% confidence interval.

Results

The ≥60 year age group presented with the highest pre-treatment Mean Parasite Density (MPD) of 3.53 mf/mg skin and a post-treatment MPD of 0mf/mg skin representing a 100% clearance rate of oral administration of chloroquine on *O. volvulus* (Table 1). The pre and post-treatment MPDs were significantly ($X^2 = 9.48$: $p<0.05$) dependent on age. The ≥60 year age group had the highest pre-treatment MPD of 4.00mf/mg skin, while the post-treatment MPD remained unchanged (Table 2). The highest pre-treatment MPD of 5.29mf/kg skin was recorded by the ≥60 year age category, while the post-treatment MPD was found to be 0mf/mg skin representing a 100% clearance rate of oral administration of doxycycline on *O. volvulus* (Table 3). The total pre-treatment MPD was observed to be 10.51mf/kg skin, while the total post-treatment MPD was 0mf/kg skin representing a 100% clearance rate. Age co-related significantly ($X^2= 10.52$: $P<0.05$) with the pre and post-treatment MPDs. The ≥60 year age group had the highest pre-treatment MPD of 5.24 mf/mg skin which remained unchanged after treatment with multivitamin tablets (Table 4). A total pre-treatment MPD of 9.91mf/kg skin was observed and remained same after treatment with multivitamin tablets. The ≥60 year age group presented the highest pre-treatment MPD of 5.29 mf/mg skin. Within weeks 1-3 of administration of doxycycline, the post-treatment MPD of the ≥60 year group was unchanged (Table 5). At week 4, the post-

treatment MPD within the ≥60 year age group dropped to 1.9 mf/mg skin. The total pre-treatment MPD was found to be 10.51 mf/mg skin. During weeks 1-3, the post-treatment MPD was the same with the pre-treatment density, while there was an insignificant reduction (X^2=3.7: p>0.05) to 3.7mf/mg skin at week 4. At weeks 5 and 6, the total post-treatment MPDs were ≥0mf/mg skin representing a significant (X^2=10.52: p<0.05) clearance rate of 100%.

Discussion

The outcome of this trial provides strong evidence in favour of doxycycline in the treatment of onchocerciasis and lends credence to the call to identify and re-purpose a drug for control programs in areas of *Loa loa* co-endemicity. However, the major challenge envisaged in current control programs is the occurrence of Severe Adverse Effects (SAEs) in co-endemic areas. While parasitized persons treated with standard dose of chloroquine presented with 100% clearance rate, evidence [6] suggests the resurgence of parasites 28 days after treatment. Furthermore, the age-dependency of parasite density highlighted by this trial warrants that a potential drug of choice must be well tolerated by the aged population.

The elevated parasite load observed with increasing age may be connected with the predisposition of the aged population to farming, fishing and other river-side activities which increase their vulnerability to the disease vector. Following oral administration of standard dose of chloroquine, there was a complete elimination of parasites across all age groups, while the parasite load of the placebo group remained the same before and after treatment. The 100% clearance rate recorded in the present study corroborates the findings of a previous study [6] which also reported a clearance rate of 100% by chloroquine phosphate on dermal *O. volvulus in vivo.* Although the previous study [6] posited that 28 days after treatment, the microfilariae density returned to its pre-treatment level and at 35 days it had increased to 121.6% of its pre-treatment value; the present study did not investigate the possibility of recrudescence and therefore could not draw reasonable comparative inferences on parasite resurgence. However, the clearance duration of ≤28 days is apparently sufficient to sustain therapeutic benefits prior to subsequent dosing. The significant (p<0.05) decline in parasite load after chloroquine therapy and the unaltered parasite load

recorded within the control group after placebo treatment with standard doses of multivitamin highlight the potential viability of chloroquine in providing therapeutic benefits in onchocerciasis control. However, the possibility of recrudescence reported by a previous study [6], as well as the toxicities associated with prolonged chloroquine therapy may adversely affect the arguments of the proponents of mass distribution of chloroquine in onchocerciasis control despite the remarkable elimination rate observed in the present trial.

Within the doxycycline treatment group, sustained and incremental parasite elimination was observed; before week 4, the parasite load remained unchanged, 35% of the parasites were cleared at week 4, while 100% clearance rate was recorded during weeks 5 and 6. Conversely, a previous study [15] had reported that only 51% of the filaria worms were alive after treatment, compared to 84% in the untreated patients, indicating a moderate but distinct macrofilaricidal activity of doxycycline at 100mg daily dose for 5 weeks. The inconsistency in results is expected because the present study examined *O. volvulus* microfilariae, while the previous study investigated the adult worms; moreover, the therapeutic doses applied in both studies were different. The potential application of doxycycline in the treatment of onchocerciasis will require a prolonged and effective drug distribution mechanism which may likely affect therapeutic coverage and compliance; however, a successful and effective mass distribution of the drug has been reported in Cameroon [16]. The outcome of the present study compares closely with a previous study [16] and demonstrates the effectiveness of doxycycline in the treatment of onchocerciasis, although the previous study additionally posited that doxycycline had the added advantage of killing adult *O. volvulus* with no toxic effect on adult *Loa loa* or their microfilariae. In areas of *O. volvulus/Loa loa* co-endemicity, the administration of ivermectin aggravates a series of Severe Adverse Effects (SAEs) initiated by the hyper-accumulation of dead *Loa loa* microfilariae. This is worrisome, and poses a great threat to the mass distribution of ivermectin, which has for long, remained the conventional method of onchocerciasis control.

Onchocerciasis and Loasis share a common ecological preponderance which accounts for the high rate of co-endemicity [17]. The choice drug for the treatment of onchocerciasis in endemic communities must be a filaricide that combines the

advantage of efficacy and selective toxicity to forestall SAEs due to *Loa loa* morbidity. In addition, prolonged administration of chloroquine has been associated with corneal deposits, posterior subcapsular lens opacity, ciliary body dysfunction, macular pigment loss, peripheral bone spicule formation, vascular attenuation, and optic disc pallor [18]. Ocular symptoms of retinopathy associated with chloroquine include blurred vision, partial loss of central and peripheral vision and in the later stage, loss of night vision [18]. Acute severe chloroquine toxicity is associated with 10–30% mortality owing to a combination of direct cardiovascular effects and electrolyte derangements with resultant dysrhythmias [19]. The short-term and long-term toxicities of chloroquine have been reported; in the long term, retinopathy is the major dose-limiting toxicity and the risk is higher with increasing age, dose, and duration of usage [20]. Furthermore, there are empirical evidences which suggest that the toxicological effects of chloroquine include cardiotoxicity which has been presented as a strong argument against recent trials to re-purpose it for the treatment of other ailments [21-23]. On the other hand, doxycycline has proved effective as a filaricide with no action against *Loa loa* microfilariae and has shown relative safety in therapeutic applications without adverse effects [4]. The present study and the findings of Akujobi *et al.* [24] have shown that parasite density and prevalence of onchocerciasis were age-dependent; becoming higher with increasing age. Therefore, mass distribution and prolonged administration of chloroquine in onchocerciasis control would be contraindicated based on subsisting proofs of toxicity among the aged population [20-23]. However, doxycycline proved effective against *O. volvulus* and is well tolerated among the aged population and patients infected with moderate intensities of *L. loa* microfilariae [25]. Although a previous study [6] had reported the occurrence of parasite recrudescence 28 days after treatment with chloroquine, the present study did not investigate this finding due to paucity of fund. In addition, the sample size of the present study was limited by some religious and cultural considerations among members of the community relative to skin extirpation.

Conclusion

The outcome of the present trial supports the exploration of doxycycline as a suitable substitute for ivermectin in the treatment of onchocerciasis in persons co-infected with

O. volvulus and *L. loa* parasites. Furthermore, it highlights the rationale and need for doxycycline to provide the much needed remedy to the threat of SAEs in co-infected persons treated with ivermectin.

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References

1. Nwoke BEB. Worms and Human Diseases. Owerri. *Alphabet Nigerian Publishers* 2009.
2. Engelkirk PG, Williams JF, Schmidt GM, Leid RW. Zoonotic onchocerciasis. *Handbook Series in Zoonoses 1982*; (2): 225-250.
3. Shelley AJ. Vector Aspects of the Epidemiology of Onchocerciasis in Latin America. *Annual Review of Entomology. 1988*; 33: 337-366.
4. Hoerauf A, Marfo-Debrekyei Y. Efficacy of 5-week doxycycline treatment on adult *Onchocerca volvulus. Parasitology Research* 2009; 104 (2): 437-447.
5. Hoerauf A, Mand S, Volkmann L, Buttner M, Marfo-Debrekyei Y, Taylor M, Adjei O, Buttner DVV. Doxycycline in the treatment of human onchocerciasis: Kinetics of Wolbachia endobacteria reduction and inhibition of embryogenesis in female onchocerca worm. *Microbes Infect*. 2003; 5(4): 261-73.
6. Guderian RH, Anselmi M, Beck BJ, Mackenzie CD, Williams JF, Proano JR, Cooper PJ. The effect of antimalarial chloroquine therapy and prophylaxis on concurrent infection with *Onchocerca vovulus* in Ecuador. *Trans R Soc Trop. Med. Hyg.* 1991; 85 (5): 634-638.
7. Ukaga CN, Ezigbo JC, Onyeka PIK, Anosike JC, Nwoke BEB. The effect of chloroquine on male worms of *Onchocerca vovulus in-vitro*. *The Nigerian Journal of Parasitology* 2003; 24: 71-76.
8. Guderian RH, Anselmi M., Cooper PJ, Chico ME. Macrofilacidal effects of chloroquine Adult on onchocerca vovulus by local infiltration of palpable onchocercal nodules. *Rev. Soc. Med. Trop.* 1997; 30(6) Uberaba.

9. Kamgno K, Djomo P, Pion SD, Thylefors B, Boussinesg MA. Controlled Trial to Assess the Effect of Quinine, Chloroquine, amodaquine and Artesunate on loa loa microfilaremia. *Am Jour Trop Med Hyg.* 2010; 82(3): 379-385.

10. Turner JD, Tendengfor N, Esum M, Johnston KL, Langley RS, Ford L, Faragher B, Specht S, Mand S, Horeauf A, Enyong P, Wanji S, Taylor MJ. Macrofilaricidal activity after doxycycline only treatment of Onchocerca volvulus in an area of loa loa co-endemity: A Randomized controlled trial. *Plos Negl Trop Dis.* 2010 4(4): e660–doi: 10 – 1371/Journal/prod.0000660.

11. Abegunde AT, Ahuja RM, Okafor NJ, Stroger JH. Doxycycline plus ivermectin versus ivermectin alone for treatment of patients with onchocerciasis. Cochrane Database Syst Rev. 2016; (1): CD011146. doi: 10.1002/14651858.CD011146.pub2

12. Burnham G, Harries A, Macheso A, Wirima J, Molyneux M. Chloroquine-induced pruritus in Malawi: lack of association with onchocerciasis. *Trans R Soc Trop Med Hyg.* 1989;83: 527–528.

13. Guderian RH, Anselmi M, Beck BJ, Mackenzie CD, Williams JF, Proano S, Jr, Cooper PJ. The effect of antimalarial chloroquine therapy and prophylaxis on concurrent infection with *Onchocerca volvulus* in Ecuador. *Trans R Soc Trop Med Hyg.* 1991; 85: 634–638.

14. Guderian RH, Anselmi M, Cooper PJ, Chico ME. Macrofilaricidal effects of chloroquine on adult *Onchocerca volvulus* by local infiltration of palpable onchocercal nodules. *Rev Soc Bras Med Trop.* 1997; 30: 469–473.

15. Hoerauf A, Marfo-Debrekyei Y. Efficacy of 5-week doxycycline treatment on adult *Onchocerca volvulus. Parasitology Research.* 2009; 104 (2): 437-447.

16. Wanji S, Tendongfor N, Nji T, Esum M, Che JN, Nkwescheu A, Alassa F, Kamnang G, Enyong PA, Taylor MJ, Hoerauf A, Taylor DW. Community-directed delivery of doxycycline for the treatment of onchocerciasis in areas of co-

endemicity with loiasis in Cameroon. *Parasites & Vectors.* 2009; 2(39)

17. Petney TN, Andrews RH. (1998). Multiparasite communities in animals and humans: frequency, structure and pathogenetic significance. *International journal of Parasitology.* 1998; 28: 377- 393.
18. Stokkermans TJ, Goyal A, Trichonas G.: Chloroquine and Hydroxychloroquine Toxicity. StatPearls Publishing, Treasure Island (FL) 2022.
19. Porta AD, Bornstein K, Coye A, Parris MA. Acute chloroquine and hydroxychloroquine toxicity: A review for emergency clinicians. *Am J Emerg Med*. 2020; 38(10): 2209–2217.
20. Doyno C, Sobieraj DM, Baker WL. Toxicity of chloroquine and hydroxychloroquine following therapeutic use or overdose. *Clinical Toxicology.* 2021; 59(1)
21. Haeusler IL, Chan XHS, Guerin PJ, White NJ. The arrhythmogenic cardiotoxicity of the quinoline and structurally related antimalarial drugs: a systematic review. *BMC Med.* 2018; 16:, 200
22. White NJ. Cardiotoxicity of antimalarial drugs. *Lancet Infect Dis* 2007; 7:549-558
23. Borba MGS, Val FAV, Sampaio VS, Alexandre MAA, Melo GC, Brito M. Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: a randomized clinical trial. *JAMA Net Open* 2020; 3 Article e208857.
24. Akujobi AU, Ukaga CN, Vincent CC, Obioma-Elemba JE, Akogu OA, Ejidike GE, Ihekaire D. Prevalence and distribution of onchocerciasis in Ahani-Achi Community, Enugu State, Nigeria. *Nigerian Journal of Parasitology.* 2017; 38(2): 149-152.
25. Turner JD, Tendongfor N, Esum M, Johnston KL, Stuart Langley R, Ford L, Faragher B, Specht S, Mand S, Hoerauf A, Enyong P, Wanji S, Taylor MJ. Macrofilaricidal Activity after Doxycycline Only Treatment of *Onchocerca volvulus* in an Area of *Loa loa* Co-Endemicity: A Randomized Controlled Trial. *PLoS Negl Trop Dis*. 2010; 4(4): e660.

Tables:

Table 1: Age-related Mean Parasite Density (MPD) of *O. volvulus* Pre and Post-chloroquine intervention.

Age (Years)	Pre-treatment MPD *O. volvulus* (mf/mg skin)	Post-treatment MPD *O. volvulus* (mf/mg skin) (% clearance)
20-29	1.02	0
30-39	1.50	0 (100)
40-49	1.65	0 (100)
50-59	1.75	0 (100)
≥60	3.53	0 (100)
Total	**9.45**	**0 (100)**
Chi square (X^2) = 9.48		
p-value= 0.023545		

Table 2: Age-Related Mean Parasite Density (MPD) of *O. volvulus* Pre and Post-Multivitamin Intervention.

Age (Years)	Pre-treatment MPD *O. volvulus* (mf/mg skin)	Post-treatment MPD *O. volvulus* (mf/mg skin) (% clearance)
30-39	0	0 (0)
40-49	1	1 (0)
50-59	1.25	1.25 (0)
≥60	4.00	4.00 (0)
Total	**6.25**	**6.25 (0)**

Table 3: Age-Related Mean Parasite Density (MPD) Of *O. volvulus* Pre and Post-Doxycycline Intervention.

Age (Years)	Pre-treatment MPD	Post-treatment MPD
	***O. volvulus* (mf/mg skin)**	***O. volvulus* (mf/mg skin) (% clearance)**
10-19	0	0 (0)
20-29	0	0 (0)
30-39	2. 00	0 (100)
40-49	1.72	0 (100)
50-59	1.50	0 (100)
≥60	5.29	0 (100)
Total	**10.51**	**0 (100)**
Chi square (X^2)=10.52		
p-value=0.014626		

Table 4: Age-Related Mean Parasite Density (MPD) of *O. volvulus* pre and post-multivitamin Intervention.

Age (Years)	Pre-treatment MPD	Post-treatment MPD
	***O. volvulus* (mf/mg skin)**	***O. volvulus* (mf/mg skin) (% clearance)**
10-19	0	0 (0)
20-29	0	0 (0)
30-39	2	2 (0)
40-49	1.00	1.00 (0)
50-59	1.67	1.67 (0)
≥60	5.24	5.24 (0)
Total	**9.91**	**9.91 (0)**

Table 5: Doxycycline therapy profile

Age (Years)	Pre-treatment MPD	Post-treatment MPD					
		Week 1	week 2	week 3	week 4	week 5	week 6
10-19	0	0	0	0	0	0	0
20-29	0	0	0	0	0	0	0
30-39	2	2	2	2	0.7	0	0
40-49	1.72	1.72	1.72	1.72	0.6	0	0
50-59	1.50	1.50	1.50	1.50	0.53	0	0
≥60	5.29	5.29	5.29	5.29	1.9	0	0
Total	**10.51**	**10.51**	**10.51**	**10.51**	**3.7**	**0**	**0**
Chi square (X^2)					**3.27**	**10.52**	**10.52**
p-value					**0.35184**	**0.014626**	