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Enhanced efficacy of sequential administration of Albendazole for the clearance of *Wuchereria bancrofti* infection: Double blind RCT

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Abstract. Till today, there is no effective treatment protocol for the complete clearance of *Wuchereria bancrofti* (*W.b*) infection that causes secondary lymphoedema. In a double blind randomized control trial (RCT), 146 asymptomatic *W. b* infected individuals were randomly assigned to one of the four regimens for 12 days, DEC 300 mg + Doxycycline 100 mg co-administration or DEC 300 mg + Albendazole 400 mg co-administration or DEC 300 mg + Albendazole 400 mg sequential administration or control regimen DEC 300 mg and were followed up at 13, 26 and 52 weeks post-treatment for the clearance of infection. At intake, there was no significant variation in mf counts ($F(3,137)=0.044$; $P=0.988$) and antigen levels ($F(3,137)=1.433$; $P=0.236$) between the regimens. Primary outcome analysis showed that DEC + Albendazole sequential administration has an enhanced efficacy over DEC + Albendazole co-administration (80.6 Vs 64.7%), and this regimen is significantly different when compared to DEC + doxycycline co-administration and control ($P<0.05$), in clearing microfilaria in 13 weeks. Secondary outcome analysis showed that, all the trial regimens were comparable to control regimen in clearing antigen ($F(3, 109)=0.405$; $P=0.750$). Therefore, DEC + Albendazole sequential administration appears to be a better option for rapid clearance of *W. b* microfilariae in 13 weeks time. (Clinical trials.gov identifier – NCT02005653)

INTRODUCTION

Diethylcarbamazine (DEC) has been the drug of choice for treatment of microfilaria (mf) carriers of *Wuchereria bancrofti (W.b)* and *Brugia malayi (B.m)* for nearly six decades. While DEC reduces mf density rapidly in vivo, it fails to clear the adult worms, which are responsible for the production of mf and initiation of lymphatic pathology. It is well known that single dose of DEC is insufficient for the complete clearance of microfilaria from the host (Chandra & Paramanik, 2008). Several studies have shown that, per oral DEC 6 mg/kg/day for 21 days or 12 days could reduce the prevalence of infection to the level of 20 to 40% (Khan *et al.*, 1998; Simonsen *et al.*, 1995; Andrade *et al.*, 1995) and more than 70% of the parasitologically cured continue to be antigen positive at 17 and 24 months post-treatment (Weerasooriya *et al.*, 1996; Meyrowitsch *et al.*, 1998). Study recruiting 729 mf carriers in India has shown that at least 4 courses of 12 days DEC were required for complete clearance of mf (Pani *et al.*, 1991). During the last two decades, efforts were made to identify the antihelminthic drugs, which could clear the microfilariae through macrofilaricidal (adulticidal) effect (Rana & Misra-Bhattacharya 2013). Albendazole, found to be effective in experimental sub-periodic *Brugia malayi* infection (Mak *et al.*, 1984) acting on beta tubulin of adult worm

(Chambers *et al.*, 2010; Nayak *et al.*, 2011), antibiotics like rifampicin, minocycline and doxycycline acting on the *Wolbachia* endosymbiont of the parasite (Taylor *et al.*, 2005a; Specht *et al.*, 2008; Taylor *et al.*, 2005b Hoerauf *et al.*, 2008), have opened new avenues for macrofilaricidal therapy as well as for short course combination therapy (Pani *et al.*, 2002; Bockarie *et al.*, 2007). Attempts are being made to identify the most suitable short course combination to clear macrofilaria (adult worm) and mf from human host. We conducted a clinical trial to study the efficacy of sequential administration of DEC + Albendazole compared to the co-administration of Albendazole or Doxycyclin with DEC in clearing the mf and antigen from human host.

MATERIALS AND METHODS

Study Participants

Potential study participants with *W.b* microfilaremia were identified through night blood surveys in 35 endemic villages of Vector Control Research Centre (VCRC) field practice areas situated in Pondicherry and nearby Tamil Nadu regions, South India. Adults with night blood microfilaria counts > 10 mf/ml by membrane filtration were invited to participate in the trial. Microfilaremic individuals with body weight less than 30 kg, treatment history of filariasis in the past 2 years or de-worming medications during the past one year, concurrent illness like hypertension, diabetes mellitus, cardiac conditions, epilepsy, renal or liver disease, psychiatric disorders and patients under rifampicin or minocycline or doxycycline therapy were excluded from the trial. Pregnant women and lactating mothers were also excluded from the trial.

Sample size

Earlier study in Egypt has shown that seven daily doses of oral DEC (6 mg/kg) and Albendazole (400 mg) could completely clear mf from 75% of the carriers at one year post-treatment (El Setouhy *et al.*, 2004). The reported clearance with DEC regimen was 25 to 40%. Therefore, to detect a difference of 40%, the required sample size was worked out to be a minimum of 35 participants in each regimen, which will yield a power of 90% with 95% confidence.

Trial regimens and placebo

Trial regimens:

DEC and Albendazole co-administration: DEC 300 mg + Albendazole 400 mg per oral single dose for 12 days.

DEC and Doxycycline co-administration: DEC 300 mg + Doxycycline 100 mg per oral single dose for 12 days.

DEC and DEC + Albendazole sequential administration: First DEC 300 mg was administrated per oral as single dose for 12 days, *sequentially* with DEC 300 mg + Albendazole 400 mg for 12 days as second pulse 30 days after initiating DEC therapy.

Control regimen:

DEC 300 mg per oral - single dose for 12 days.

Study design and randomization

The study was carried out as a double blind randomized controlled clinical trial. The Scientific Advisory Committee (SAC) of VCRC approved the study and the Institutional Human Ethics Committee (IHEC) of VCRC cleared the proposal following Indian Council of Medical Research (ICMR) guidelines. All eligible participants were divided into blocks of size four and within each block, individual randomization irrespective of the gender and blood microfilaria count was done to have almost equal number of participants in each regimen. After obtaining informed written consent, a medical officer recruited the eligible participants in the trial during the period February 2009 and September 2010.

Investigations

Biological sample for investigation: Trained technicians collected venous blood samples between 20.00 and 22.00 hours from each participant at intake (pre-treatment) and at pre determined intervals following therapy for biochemical, parasitological and serological examination. Biochemical parameters were carried out at intake using identi / aptec / Raichem reagents in Olympus AU 400 automated analyzer.

Methods for parasitological parameters: 1 mL of venous blood was subjected to membrane filtration (with 5 micron membrane filter, Millipore, type TMTP) following standard procedure (Chandrashekar *et al.*, 1984) and the mf number was counted by microscopic examination of stained filters. For quantification of circulating filarial antigen, commercially available Og4C3 enzyme linked immunosorbant assay kit (Tropical Biotechnology Pvt. Ltd. Townsville, Australia) was used following manufacturer's instructions. In addition, Immuno-chromatography card test (AD12-ICT) for the detection of 200 kDa secretary filarial antigen (Binax, Portland, ME) was performed at intake and one year post-treatment.

Treatment and follow-up

Under the supervision of a medical officer, trained health workers provided all 12 days therapy to the participants at their door steps for both the pulses of treatment. Fig. 1 depicts the recruitment, randomization, and follow-up of this study. After initiating therapy, one clinical nurse visited the study participants every day to record the symptoms of adverse reactions and reported to the medical officer for treatment.

Defined end-points of the study

The primary endpoint was defined as the complete clearance of mf in 80% of the participants at 13 weeks post-treatment. The secondary end-point was defined as complete clearances of mf and antigen at 26 weeks and 52 weeks post-treatment respectively. The follow-up beyond 52 weeks (360 days) was not considered as it was beyond the scope of this study to differentiate resurgence or re-infection.

Statistical analysis

Statistical analyses were performed with a statistical software package, IBM SPSS, Version 19.0. The baseline characteristics were compared across the four treatment regimens using analysis of variance (ANOVA). Chi-square test was applied to see the significant variation in mf clearance pattern among the four drug regimens at each post-treatment time point. Mf density and antigen units were subjected to ANCOVA test after normalizing the data by taking logarithmic transformation. To know the efficacy of clearing 200 kDa antigen by drug regimens and also to know the association between the adverse reactions and the treatment regimens, Pearson chi- square test was performed.

RESULTS

Baseline Characteristics, adherence to treatment and follow-up

In total, 254 mf carriers were assessed by a medical officer and 146 eligible participants were recruited in the trial. Five participants withdrew before the completion of first pulse therapy due to mild adverse reactions. These five participants were included in the analysis of adverse reactions during the first pulse therapy and excluded from other analyses. Baseline demographic characteristics and the parasitological parameters of the 141 participants who had completed treatment are given in Table 1. There was no significant variation in baseline mf counts ($F(3,137) = 0.044$; $P=0.988$) and antigen levels ($F(3,137)=1.433$; $P=0.236$) between the regimens. We could achieve at least 87% case holding in all the follow-up and the drop out was mainly due to the non availability of the participants at the time of visits and social stigma. There was no significant difference in drop-out rate between the regimens in all the three follow-up ($P>0.05$).

Adverse reactions

All the participants received two pulses of treatment which includes placebo during second pulse of therapy except in DEC + Albendazole sequential administration. It was observed that during pulse one therapy, 56.4%, 44.7%, 54.5% and 41.7% participants reported one or more adverse reaction in DEC + Albendazole co-administration, DEC + doxycycline co-administration, DEC + Albendazole sequential administration and DEC control regimen respectively. Similarly, during the second pulse of therapy, 13.5%, 13.2%, 18.8% and 14.7% participants reported

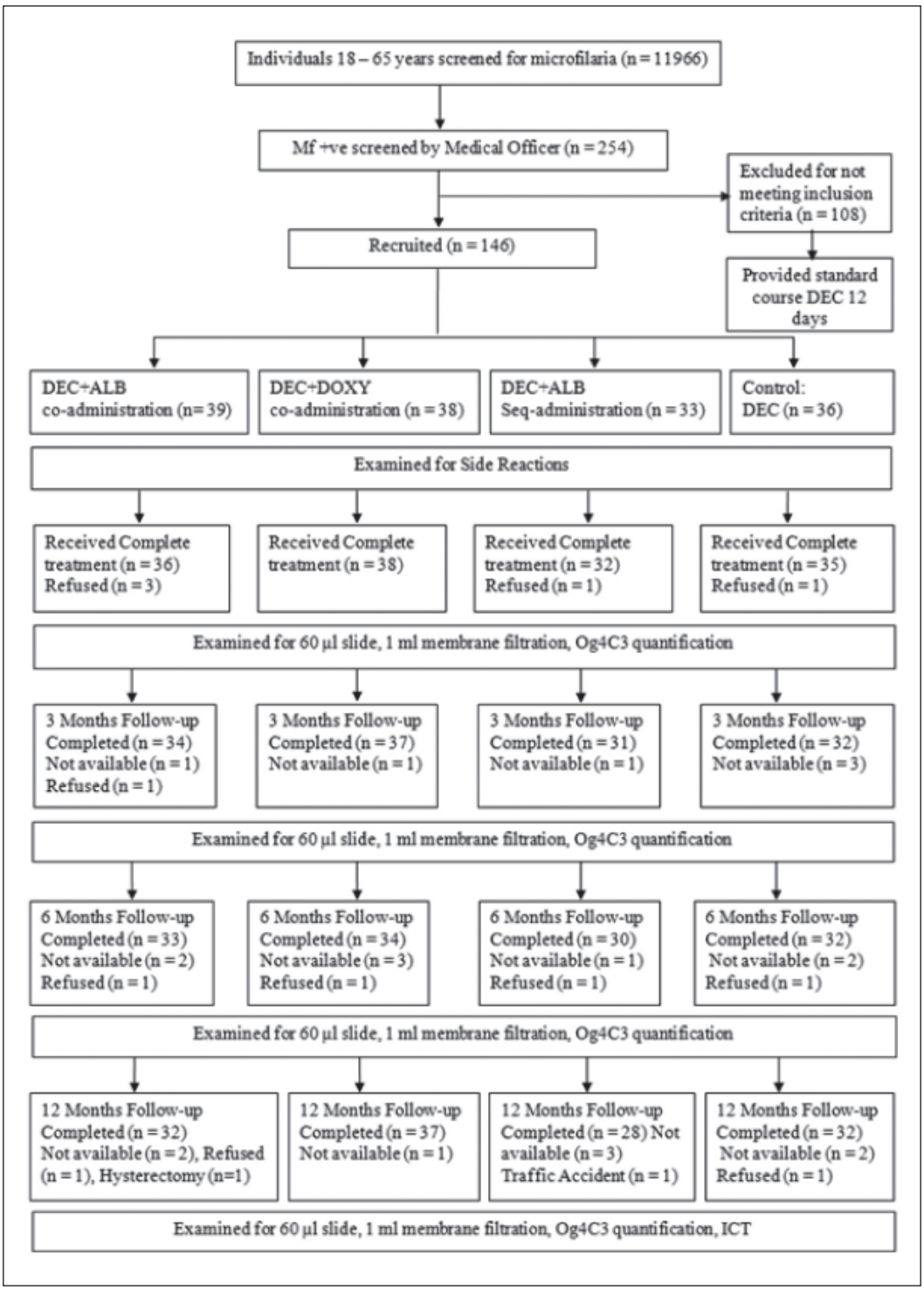

Figure 1. Screening, Randomization, and Follow-up of the Study Participants

A total of 146 *W.b* mf positives were recruited in the study by block randomization and five of them dropped out from treatment due to adverse reactions. All the 141 participants who had completed the treatment were approached at 13, 26 and 52 weeks post-treatment follow up for studying the outcome parameters. We were able to achieve at least 87% case holding at all three post-treatment follow up.

Table 1. Baseline characteristics of 141 participants according to study group†+

Characteristics	DEC+ALB Co-administration N=36	DEC+DOXY Co-administration N=38	DEC+ALB Sequential administration N=32	Control: DEC N=35
Age – Years				
Mean	35.8±10.2	35.5±11.9	35.0±9.9	36.1±11.8
Range	33	44	37	39
Female sex no. (%)	10(27.8)	9(23.7)	11(34.4)	10(28.6)
Mf Count (60µl Slide)				
Mean	17.1±24.7	9±9.1	12.9±13.5	13.3±18
	7.74	5.28	7.81	7.29
Range	1-130	1-38	1-51	1-82
Mean (Log)	0.89±0.57	0.72±0.49	0.89±0.45	0.86±0.48
Mf Count (1 ml#)				
Mean	366.3±418.8	273.9±274.7	372.5±398.0	32.7±322.7
	178.2	161.4	170.71	164.8
Range	19-1600	15-1018	10-1302	11-1330
Mean (Log)	2.26±0.57	2.21±0.48	2.24±0.62	2.22±0.52
Og4C3 Ag (Units*)				
Mean	12814±12704	11727±11710	14070±17941	8829±10365
	7634	5669	5774	3826
Range	840-51715	171-44718	513-59502	75-44110
Mean (Log)	3.88±0.48	3.75±0.63	3.76±0.63	3.58±0.69
ICT Positive no. (%)	36 (100)	38 (100)	32 (100)	35 (100)

† plus –minus values are means ±SD
# By filtration of one ml of blood through Millipore membrane.
* Antigen units were calculated as per the instructions of manufacturer's of Og4C3 kit using semi log parameter
No significant difference between regimens at base line mf count (P = 0.9888) and Og4C3 Ag level (P = 0.236)

one or more adverse reaction in the above order of drug regimen is given in Table 2. There was no significant difference in adverse reactions between the regimens during first pulse (Pearson Chi-Square 2.31; P = 0.511) and second pulse therapy (Pearson Chi-Square 0.52; P = 0.914).

Primary outcome analysis

Early mf clearance

It was observed that at 13 weeks post-treatment, 64.7%, 24.3%, 80.6% and 25% of the mf carriers completely cleared mf in DEC + Albendazole co-administration, DEC + doxycycline co-administartion, DEC + Albendazole sequential administration and DEC control regimen respectively (Fig. 2). The clearance of mf at 13 weeks post-treatment was significantly higher in DEC + Albendazole co-administration and DEC + Albendazole sequential administration compared to DEC + doxycycline co-administration and DEC control regimen (P<0.05). DEC + Albendazole sequential administration has an enhanced efficacy over DEC + Albendazole co-administration (80.6 Vs 64.7%), and the observed clearance rate between DEC + Albendazole sequential administration (80.6%) and DEC control regimen (25%) resulted in >90% power with 33 and 36 participants in the respective drug regimen.

Table 2. Side reactions reported by participants in each regimen during the first and second pulse of treatment

Treatment	DEC+ALB Co-administration	DEC+DOXY Co-administration	DEC+ALB Sequential administration	Control: DEC
First Pulse				
Received-no. (N=146)	39	38	33	36
Side Reaction Duration				
1-3 days- no. (%)	11(28.2)	12(31.6)	12(36.4)	11(30.6)
4-6 days- no. (%)	7(17.9)	5(13.2)	5(15.2)	3(8.3)
7 days and above –no. (%)	4(10.3)	0(0)	1(3.0)	1(2.8)
Second Pulse #				
Received-no. (N=141)	37	38	32	34
Side Reaction Duration				
1-3 days- no. (%)	3(8.1)	4(10.5)	2(6.2)	4(11.8)
4-6 days- no. (%)	2(5.4)	1(2.6)	4(12.5)	1(2.9)
7 days and above –no. (%)	0(0)	0(0)	0(0)	0(0)

Five participants did not receive second pulse
There was no significant difference in side reactions between the regimens.
First pulse: Pearson Chi-Square 2.31; P = 0.511
Second pulse: Pearson Chi-Square 0.52; P = 0.914

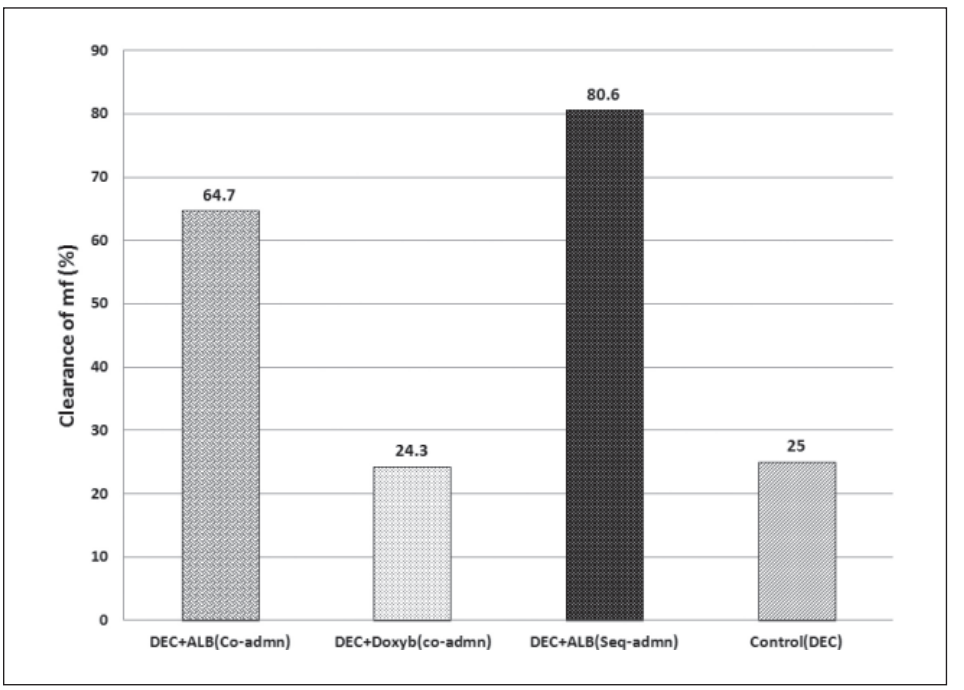

Figure 2. Primary outcome analysis: Complete clearance (% of participants) of *W.b* mf at 13 weeks post-treatment follow up. Complete clearance was significantly higher in DEC + ALB co-administration and DEC + ALB sequential administration compared to DEC + doxycycline co-administration and the control regimen DEC (P<0.05)

Secondary outcome analysis

Mf clearance at 26 weeks and 52 weeks

At 26 weeks post-treatment, there was a significant (P<0.05) difference in clearance rates (% participants completely clearing mf) among DEC + Albendazole co-administration and DEC + Albendazole sequential administration compared to that of DEC control regimen (Fig. 3A), whereas at 52 weeks post-treatment, the clearance pattern among three trial regimens, DEC + doxycycline, DEC + Albendazole sequential administration and DEC + Albendazole co-administration did not differ significantly (P>0.05). Further, in all the three time points, there was no significant (P>0.05) difference in mf clearance pattern between the regimens that involved Albendazole. However, at 52 weeks post-treatment, the observed difference in clearance rates between DEC + Albendazole sequential administration and DEC control regimen resulted in more than 90% power.

Change in mf density

The estimated marginal mean mf density (log values) at baseline in DEC + Albendazole co-administration, DEC + doxycycline co-administration, DEC + Albendazole sequential administration and DEC control regimen were 2.25, 2.23, 2.31 and 2.21 respectively. Mf density reduced to 0.30, 1.00, 0.18 and 1.06 at 13 weeks post treatment, 0.15, 0.65, 0.10 and 0.83 at 26 weeks post-treatment and 0.07, 0.16, 0.01 and 0.52 at 52 weeks post-treatment in the above order of regimens respectively (Fig. 3B). Pair treatment regimens comparison of means showed that the level of reduction did not differ significantly between the regimens DEC + doxycycline co-administartion and DEC control regimen and between Albendazole co-administration and DEC + Albendazole sequential administration. The level of reduction in mf count was highly significant (P<0.001) in DEC + Albendazole sequential administration compared to DEC + doxycycline co-administration and DEC control regimens.

Antigen clearanceCirculating filarial antigen by Og4C3 assay and by AD12-ICT

Fig. 3C depicts changes in antigen level in each treatment regimen at different time points of follow-up. The estimated marginal mean Og4C3 antigen level (log values) at baseline in DEC + Albendazole co-administration, DEC + doxycycline co-administration, DEC + Albendazole sequential administration and DEC control regimen were 3.78, 3.81, 3.78 and 3.54 respectively. Og4C3 antigen level reduced to 3.33, 3.43, 3.60 and 3.40 at 13 weeks post-treatment and 3.28, 3.44, 3.60 and 3.40 at 26 weeks post-treatment and 2.89, 2.95, 2.93 and 2.90 at 52 weeks post-treatment in the above order of regimens respectively. Though there was a reduction in antigen level at 52 weeks post-treatment, none of the three tested regimens was significantly different from the control regimen in clearing the antigen ($F(3, 109) = 0.405$, $P = 0.750$). At 52 weeks post-treatment, we also performed ICT on a proportion of participants in each regimen, depending on the availability of the ICT cards and it was observed that 15/22 (68.2%), 24/28 (85.7%), 17/23 (73.9%) and 15/22 (68.2%) of *W.b* infected individuals were positive in the above order of regimens respectively and there was no significant variation in ICT prevalence between the regimens (Pearson Chi-Square = 2.979, p = 0.424).

DISCUSSION

The natural history of human filarial infections leading to development of disease has been a subject of intense debate. In the absence of a specific tool to identify the infected individuals at risk of developing the clinical manifestations, it is an ethical responsibility of the health provider to offer an effective treatment to clear the infection, preferably a short course treatment as compliance is the major concern in filariasis management (Babu *et al.*, 2004; Njomo *et al.*, 2012). However, earlier attempt with seven days of DEC and Albendazole co-

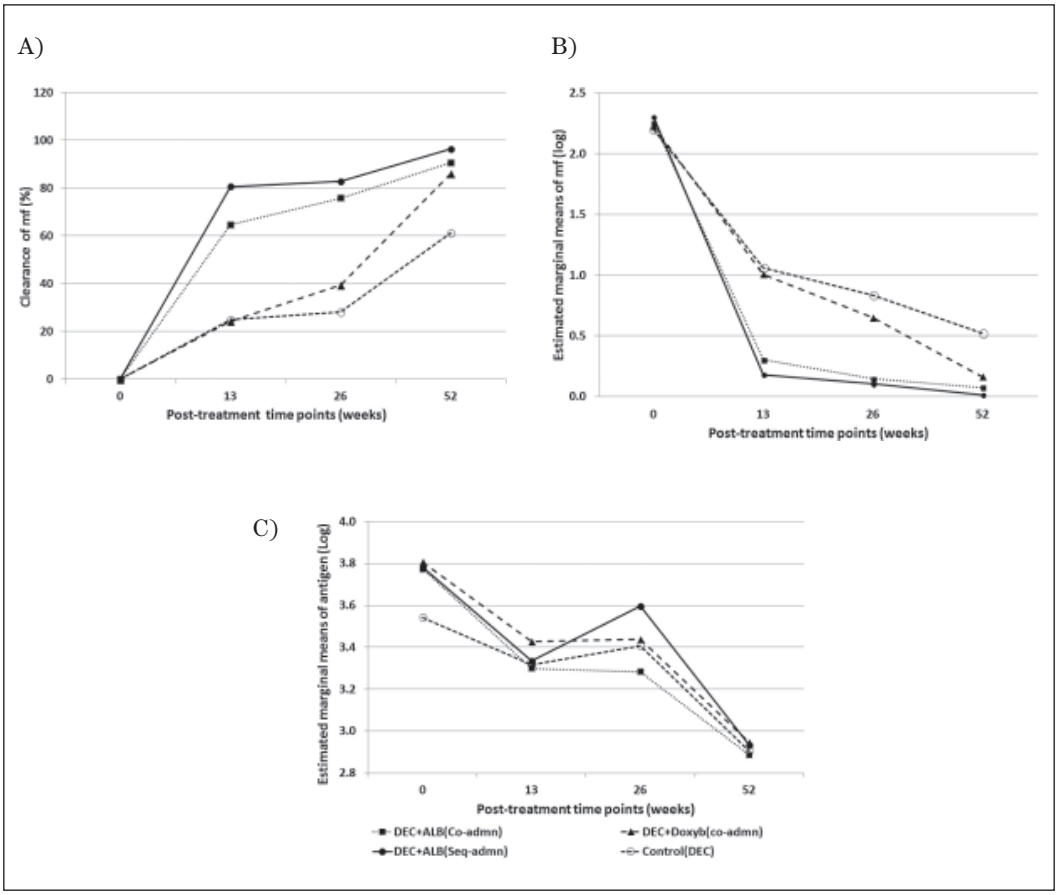

Figure 3. Secondary outcome analysis: Fig. 3A shows complete clearance (% of participants) of *W.b* mf at 13, 26 and 52 weeks post-treatment. At 26 weeks, the mf clearance rates were significantly higher in DEC + ALB co-administration and DEC + ALB sequential administration compared to DEC + doxycycline co-administration and the control regimen ($P<0.05$). At 52 weeks, the mf clearance rate of DEC + Doxycycline co-administration improved 39.4% (at 26 weeks) to 86.1% indicating late killing effect. Fig. 3B shows the changes in mf density at 13, 26 and 52 weeks follow up and change in Og4C3 antigen level at 26 weeks and 52 weeks follow up. At 52 weeks post-treatment, the level of reduction in mf density was highly significant ($P<0.001$) in DEC + Albendazole sequential administration compared to DEC + doxycycline co-administration and DEC control regimens. Fig. 3C shows changes in circulating filarial antigen level by Og4C3 assay at 13, 26 and 52 weeks follow up. Tough there was a reduction in antigen level at all three post-treatment follow up, none of the three tested regimens was significantly different from the control regimen in clearing the antigen ($F(3, 109) = 0.405$, $P = 0.750$).

administration was not convincing in terms of clearing mf in a short span of 90 days (El Setouhy *et al.*, 2004). Ultrasound findings demonstrated 88% adult worm clearance at 14 months, following eight weeks doxycycline treatment (Taylor *et al.*, 2005). Turner et al observed that 3-weeks course of doxycycline treatment, in combination with standard anti-filarial therapy is more effective in inducing a long-term amicrofilaremia than standard treatment alone (Turner *et al.*, 2006). However, in our experience we observed a steep reduction in microfilaria count at 30 days post-treatment irrespective of a single dose or combination therapy (Pani *et al.*, 2002). Field trials have shown that 4 weeks doxycycline could completely eliminate adult worm nests from scrotal lymphatics in 100% of the infected at 18 months follow-up (Debrah *et al.*, 2011) and 3 weeks doxycycline therapy with single dose of DEC at 4 month post-treatment could

completely clear microfilaria, adult worm nests and reverse lymphatic pathology at 12 months post-treatment (Mand *et al.*, 2009). Therefore, to augment the parasite clearance, it was envisaged to intervene with the sequential administration of DEC and Albendazole at 30 days following the standard DEC therapy. In order to minimize the doxycycline duration, we preferred DEC co-administration. The primary outcome was the complete clearance of mf in at least 80% of the carriers by 13 weeks and the secondary outcome was complete clearance of mf and antigen at 26 and 52 weeks post-treatment respectively. By macro-filaricidal effect, the trial regimens were expected to clear the antigen from the host by one year. Our results have established that Albendazole sequential administration has an edge over co-administration therapy (80.6% vs 64.7%), in clearing mf in a short span of 13 weeks. In contrast, standard DEC treatment and DEC + doxycycline co-administration for 12 days could clear mf only in 25% of the carriers at 13 weeks post-treatment. However, co-administration as well as sequential administration seemed to have similar partial killing effect on adult filarial worms, based on both Og4C3 and 200 kDa secretary antigen (ICT) assays at 52 weeks post-treatment. Though there was reduction in Og4C3 antigen level, more than 65% of the treated continue to be antigen positive by both the antigen assays. This finding was similar to the treatment results of filariasis with multiple high dose of Ivermectin and DEC and aggressive prolonged DEC therapy (Ismail *et al.*, 1996; Freedman *et al.*, 2001) and different regimens of doxycycline with slow killing effect in onchocerciasis (Debrah *et al.*, 2006; Hoerauf *et al.*, 2009).

Twelve days Albendazole or doxycycline co-administration and Albendazole sequential administration were well tolerated by the study participants and they did not experience severe adverse reactions. As observed in other trials (Turner *et al.*, 2006; Gayen *et al.*, 2013), the adverse reactions lasting for one to seven days were mild and responded to symptomatic treatment. We observed that a sizable proportion of the participants (13.2 to 14.7%) received placebo during second pulse reported adverse reactions indicating psycho-somatic effect. The focus of current and future research in filariasis treatment is to define the minimum length of time necessary to completely eliminate mf from the host and to achieve macrofilaricidal efficacy comparable to that of 8- or 6-weeks course of doxycycline treatment. We preferred 13 weeks effect as the end point, as this could really reflect the drug effect. Our study has shown that sequential administration of DEC and Albendazole combination 30 days following the standard DEC course for 12 days could completely clear mf in more than 80% of the infected in a short span of 13 weeks. In addition, Albendazole is a preferable therapy in children below ten years as compared to doxycycline. Therefore, sequential administration could be considered as an effective and safe short course therapy for individual mf carrier treatment. As a large proportion of the trial participants were females and the trial was carried out in rural community settings, ultrasound examination was not carried out to investigate the effect on adult worm nests. However, we carried out both Og4C3 antigen assay and ICT for AD12 antigen to know the effect on the adult worms and the results showed that this strategy is not an effective macrofilaricidal option for *W. bancrofti* infection as seen in other clinical trials involving doxycycline therapy. Perhaps, effective drug concentration is not achieved or the host immune system is not effectively modulated to kill the adult worms or antigen clearance requires completely a different mechanism. Further research is needed with alternative antibiotics or with antibiotic plus novel anti-filarial drug combinations for rapid macrofilaricidal effect which can clear adult worm within short duration of time, adopting uniform end points. Differences in end points may lead to confusions and the real benefit of right combination or strategies will not reach the public health programme.

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