DOXYCYCLINE ULTRA EARLY TREATMENT TRIAL (DUETT): A RANDOMIZED CONTROLLED TRIAL PROTOCOL FOR THE TREATMENT OF COVID-19

Taqi Hashmi F[*,1], **Maysaa Ashkanani**[*], **Miaaz M Zidan**[**], **Lahoucine Naim**[*], **Afshan Hasan**[*] **and Mohamed Nourelsabah Metwally**[*]
[*]PO Box 26555, Rawdat Alkhail Health Center, Primary Health Care Corporation, Doha, Qatar., [**]26999, Sidra Medicine, Doha, Qatar.

ABSTRACT The study aim is to compare doxycycline (a tetracycline) versus placebo for the treatment of patients with suspected COVID19 caused by SARS-CoV-2 (a positive single strand RNA virus) in an ultra early timeframe starting at the time of presentation of a patient at primary health care centers prior to a confirmed PCR result and ending at day seven or two serial negative PCR results are found whichever is earlier. The aim is to abort viral replication, reduce morbidity and mortality, and reduce the R0 of infection transmission.
The following arguments support a biologically plausible use of tetracycline for the treatment of COVID19: (1) Metalloproteases (MMPs) are upregulated in human lung tissue in inflammatory lung disease (Greenlee et al. 2007), murine studies show increased expression of MMPs due to coronavirus infection (Zhou et al. 2005). Tetracyclines are known to chelate zinc from MMPs and inhibit MMP function (NYAS Publications). (2) Tetracyclines inhibit the replication of positive single strand RNA viruses such as West Nile virus replication (Michaelis et al. 2007) and Dengue virus replication in tissue culture (Yang et al. 2007). (3) Doxycycline decreases the inflammatory cytokines such as IL-6, IL-1β, and TNF within 3 days reaching a peak effect at 7 days(Castro et al. 2011). Doxycycline is therefore a treatment option that may be able to inhibit SARS-CoV-2 replication, morbidity and mortality and transmission.

KEYWORDS COVID19, Doxycycline, Primary care, Treatment

Introduction

Coronavirus disease 2019 (COVID19) is a virally transmitted disease whose etiological agent is the positive sense of ribonucleic acid (RNA) virus named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus was identified as the causative agent of COVID19 in January 2020 [1]. It has caused a global pandemic with 30.6 million and 950,000 deaths as of 20 September 2020[2]. There is a pressing need to find an intervention that can be used as a treatment option and decrease viral carriage to limit transmission.

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[1]IPO Box 26555, Rawdat Alkhail Health Center, Primary Health Care Corporation, Doha, Qatar; Email: thashmi@phcc.gov.qa

The following arguments support a biologically plausible use of tetracycline for the treatment of COVID19:

1. Metalloproteases (MMPs) are upregulated in human lung tissue in inflammatory lung disease [3], murine studies show increased expression of MMPs due to coronavirus infection[4]. Tetracyclines are known to chelate zinc from MMPs and inhibit MMP function[5].

2. Tetracyclines inhibit the replication of positive single-strand RNA viruses such as West Nile virus replication[6] and Dengue virus replication in tissue culture[7].

3. Doxycycline decreases the inflammatory cytokines such as IL-6, IL-1β, and TNF within three days, reaching a peak effect at 7 days[8]. Doxycycline is, therefore, a treatment option that may be able to inhibit SARS-CoV-2 replication, morbidity and mortality and transmission.

Methods

Aim

The randomized DUETT study's aims to evaluate the effects of early treatment with doxycycline for patients with suspected cases of COVID19 with the primary aim of reducing hospital admission in the first 21 days from disease onset.

Study design

DUETT is a two-armed randomized controlled trial to be run in primary care front-line centres dealing with suspected COVID19 cases that have been assigned to swab patients as part of nationwide screening. The aim is to include a sufficient number of patients with suspected COVID19 to adequately power the study.

Inclusion criteria

Patients eligible for the criteria are:

- Those who are 18 years and older
- All genders
- Newly diagnosed as a suspected case of COVID19

Exclusion criteria

Patients meeting the following criteria will be excluded:

- Positive SARS-CoV-2 PCR prior to enrollment into the study.
- Unable to provide consent including those under guardianship or trusteeship or in safeguard of justice
- Needing immediate hospitalization for any medical reason
- Lactose-intolerant patients
- More than 3 days of clinical symptoms at the inclusion visit
- History of allergy to tetracyclines
- Pregnant or lactating women
- Participating in another clinical trial
- Photosensitive skin pathology
- Treated with anticoagulant
- Treated with oral retinoids: isotretinoin, alitretinoin, acitretin
- Treated with vitamin A
- Treated with systemic antibiotics for the duration of treatment
- Treated with barbiturates, carbamazepine or phenytoin
- Treated with chloroquine, hydroxychloroquine, remdesivir, ganciclovir, acyclovir, ribavirin, lopinavir-ritonavir.

Recruitment and allocation

We will approach health care networks which are responsible for initial testing of patients with suspected COVID19. A researcher will be assigned to each site to train staff on obtaining consent, data collection and the required intervention. A set number of intervention packs which are serially numbered at source either containing the active medication or a placebo. Patients will be allocated using stratified block randomization in a 1:1 ratio using a length of 4 and 6 randomized blocks lengths with stratification based on gender, age and site of inclusion. This will be done by a software program with a masked seed for randomization. All researchers, clinicians, patients and block sizes will be masked.

Intervention

The trial will have two arms, an interventional and placebo arm. The interventional arm will be treated with a three-dose regimen of doxycycline as follows: day 1 600 mg loading dose on initial clinical suspicion of COVID19, and for patients with a positive result or a negative result with high clinical suspicion will have two further doses on day 2 and day 3 400 mg/day for two further days. The placebo arm will be treated with the same dosing regimen at the same time. Patients who present to the health centre and are suspected of having COVID19 based on a clinical scoring system will be tested with oropharyngeal and nasopharyngeal swabs for reverse transcription-polymerase chain reaction (RT-PCR) testing for SARS-CoV-2. RT-PCR will be conducted daily for seven days (Day 1-7) with the Ct values being recorded of all results. They will be assigned to quarantine and will be monitored for clinical deterioration. Patients with clinical deterioration will be admitted to secondary care hospitals through their normal health care provider pathway.

Follow-up care during and after active treatment

Day 8-14: Patient records will be monitored daily by telephone to assess their clinical condition and admission to hospital.

Day 15-21: Patient records will be monitored for admission to hospital for a further 7 days to record any interaction with the health services using the centralized health care information system. A final telephone call will be made on day 21 to the patient to assess their clinical condition and record any unrecorded medical events.

Intervention training

Each regional and local health centre will have an assigned number of staff for research as outlined in Table 1. Assigned staff will be trained in consenting and following up patients, entering data into the trial website and how to dispense the assigned treatment intervention. Each registered nurse will be required to have more than 5 years of clinical experience. The DUETT study team will offer a 4 hour training program regarding consent ethics, masking, data recording, supportive care, recognizing alarm symptoms and details of the DUETT study.

Primary outcomes

1. Percentage of patients with hospital admission Day 1 to 21

Secondary outcomes

1. The median time to reach a RT-PCR Ct value of ≥ 30 for the E gene.
2. The median time to a 75% or greater improvement in the clinical symptom score (as defined in Table 2).
3. The percentage of cases who had an initial negative RT-PCR result subsequently had a subsequent positive RT-PCR result up to day 21.
4. Number of Adverse Events in both arms Day 1 to 21
5. Total duration of hospitalization Day 1 to 21
6. Percentage of patients requiring ventilatory assistance Day 1 to 21

Table 1 Assigned Roles for Research Staff.

Title	Role	FTEs
Research Team at Regional Level		
Physician Lead	Directs, organizes and trains research teams at both the regional and local level. Coordinates data collection, analysis, write up and publication of data. Will chair weekly meetings to assess trial status and changes or updates to Standard Operating Procedures.	1
Nurse Research Coordinator	Manages training of nursing staff to recruit, take patient consent, collect consent and record recruited patients in the electronic database and scan and post consent forms to the Research Coordinator, recall trial patients for follow up and monitor patients lost to the trial. Write the Standard Operating Procedures for all research staff and update any SOPs on a regular basis.	1
Pharmacy Research Coordinator	Manages the purchase, preparation and distribution of the intervention and placebo intervention medication and the masked randomization lists. Follows up all reported Adverse Drug Reactions	1
Research Assistant	Sets up and maintains an electronic database to collect and compile all trial data from all sites on a daily basis. Calls trial patients for follow-up and monitors patients lost to the trial. Track the end of medication adherence. Answer or escalate patient queries as per the given Standard Operating Procedures.	3
Biostatistician	Weekly analysis of data and publication of interim reports and final analysis prior to publication.	0.5
IT	To set up the required system to collect data and troubleshoot as required.	1
Research Team at Health Centre Level		
Nurse Coordinator	Recruits patients for the trial. Delivers instructions to the patient to allow for informed consent. Records and collects patient consent in the electronic database. Coordinates delivery of intervention or placebo to the patient and instructs patients on dosage, administration and future follow-up.	1 assigned nurse per Health Centre. FTE allocation is proportional to hours worked and regular duties performed.

7. Percentage of deaths related to SARS-CoV-2 infection Day 1 to 21

Data collection

Day 1-7: the consent, daily clinical score RT-PCR result will be collected. All patients who have been hospitalized or dropped out of the trial will also be collected. Data collection will be done via a fixed web portal with all data entries time-stamped and resulted masked from researchers until the trial is closed.

Day 8-14: A daily clinical score will be recorded, and a number of hospital admission or deaths will be recorded. The score is given in Appendix A.

Day 15-21: Patient records will be monitored for admission to hospital or death.

Adherence

Patients on day 3 will be asked to send a photograph of their medication packets to a centralized number and a count of unused medication will be done.

Statistical analyses

The statistical analysis will be performed on an intention-to-treat analysis on all randomized patients. Categorical baseline variables such as gender will be shown as frequencies or percentages. Continuous variables such as the number of hospital admissions in the time period of the study, time to randomization from symptom onset and time to negative conversion from the onset of therapy will be shown by calculating means or medians. The comparison of patients who complete the study and those who do not will be compared by Pearson's Chi-square analyses for categorical variables and by T-tests for continuous variables.

We will use a log-rank test to calculate the significance of a difference between the Kaplan-Meier plots of the overall probability of remaining RT-PCR <30 against our defined event (reaching a RT-PCR Ct value $\geq$ 30 for SARS-CoV-2) between the treatment and placebo group. A Cox model will be used to estimate the hazard ratio as a size of the effect. A hazard ratio of the intervention to the placebo group of less than one will indicate that the rate of conversion of patients from an infective to the non-infective state was higher in the intervention group compared to the control group. Patients who do not achieve the cut-off point by the end of the study will be considered right-censored.

Sample size

We have assumed a hospitalization to case ratio rate of 15% +/- 5% with an expected clinical reduction of 10%. Using the power of 80% and an alpha of 5%, at least 348 patients are required. Accounting for an estimated dropout rate of 15% a total of 400 patients are required. [9]

Discussion

Hospital-to-case Ratio The following published studies and datasets are showing the hospitalization to positive case ratios from different populations and health systems. Data from England, the United Kingdom from the National Health Service show that the average hospitalization to case ratio was 9.29% (12,205[10] out of 131,358 cases[11] over 70 days from 27 April to 5-July 2020. A Chinese study showed a hospital admission rate across 31 provincial regions of China in 575 hospitals of 13.8% (1590 out of 11791 cases) as of 31 January 2020. [12]. State-level data from New York shows an average hospitalization to case ratio of 22.4% (89,995 out of 401,706 cases) as of 12 July 2020. [13] A study of 463 serially attending COVID19 patients attending a five-hospital and 9 emergency department integrated system serving metropolitan Detroit by Henry Ford Health System (HFHS) in Southeast Michigan serving a large urban, mostly African American population in metropolitan Detroit, had a hospitalization rate of 76.7% (355 out of 463 patients), of which dyspnea was present in 60.9% (282 patients). [14] The data shows a wide degree of variation in the hospitalization to case ratio (9.29% - 76.7%). The English and Chinese health systems are state-run, while the USA data represents data from a system where the provision of healthcare is predominantly supplied by the private sector. This, in addition to the ethnicity of the populations served, are likely contributing factors to this wide variation.

RT-PCR Ct value of $\geq$ 30

The primary outcome cut off the definition of a RT-PCR Ct value of $\geq$ 30 for the E gene has been set based on studies showing viral infectivity of cell culture lines falls to zero at a RT-PCR Ct value for the E gene of 34 or greater[15] and 24 or greater[16].

Doxycycline safety

Doxycycline is absorbed almost completely (95%) when administered orally[17]. A single dose of 600 mg showed no significant difference in tolerability to placebo and maintained a serum concentration of 1.35 micromol/L at 48 hours. [18] A single dose of 600 mg has been used in patients with uncomplicated gonococcal infections [19] and this dosing is included in its datasheet by the New Zealand Medicines and Medical Devices Safety Authority. [20] A dosing regimen of 200mg twice daily in a small study given 21 days reported no cessation due to side effects. Patients had an average doxycycline serum concentration of 5.8 (3.6 - 8.6) micrograms/ml after 21 days. [21]. A single 400 mg dose of doxycycline was given to patients with a mean age of 50 years for an average of 10.8 days and was well tolerated. [22] Doxycycline is eliminated by non-renal mechanisms with 30-40% eliminated by renal mechanisms, and the rest is through hepatic and intestinal clearance. In the presence of severe renal insufficiency, non-renal elimination increases maintaining serum levels and half-life of doxycycline. [17] Assuming doxycycline's plasma concentration-time profile follows a one-compartment model with first-order absorption and elimination with the following variables: fractional bioavailability 1.16, absorption rate constant ka= 0.414/hour, elimination rate constant ke= 0.076/hour, the volume of distribution = 49 litres, the predicted serum value at 51 hours with the three doses proposed regime will be 5.18 micrograms/ml. This is below the measured average serum concentration of 5.8 micrograms/ml shown to be safe in a previous study[21].

Given the above evidence, the dosage and regimen of doxycycline used in the trial is considered to be tolerable and safe.

Conclusions

The DUETT trial will provide primary care level data to assess the use of a potential treatment for COVID-19 at an ultra stage of the disease. Results of this study could help patients, clinicians and public health officials assess in dealing with the COVID-19 pandemic.

Notes

Appendix A

Table 2 Clinical Symptom Score.

Clinical Score for Suspected or Proven COVID19 Patients	
Fever >37.8	1
Cough	1
Myalgia	1
Feeling Cold	1
Sore throat	1
Fatigue	1
Shortness of Breath	2

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This study received no fund.

Conflict of interest

There are no conflicts of interest to declare by any of the authors of this case report.

References

1. Edward C. Holmes, Novel 2019 coronavirus genome [Internet]. Virological. 2020 [cited 2020 May 30]. Available from: http://virological.org/t/novel-2019-coronavirus-genome/319
2. Coronavirus disease (COVID-19) Situation Report, [Internet]. [cited 2020 Sep 23]. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200921-weekly-epi-update-6.pdf?sfvrsn=d9cf9496_6
3. Greenlee KJ, Werb Z, Kheradmand F. Matrix Metalloproteinases in Lung: Multiple, Multifarious, and Multifaceted. Physiol Rev. 2007 Jan;87(1):69.
4. Marten NW, Zhou J. The Role of Metalloproteinases in Corona Virus Infection. In: Experimental Models of Multiple Sclerosis. Springer, Boston, MA; 2005. p. 839–48.
5. NYAS Publications [Internet]. The New York Academy of Sciences. [cited 2020 13 June]. Available from: https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.1999.tb07697.x

6. Michaelis M, Kleinschmidt MC, Doerr HW, Cinatl J. Minocycline Inhibits West Nile Virus Replication and Apoptosis in Human Neuronal Cells. J Antimicrob Chemother [Internet]. 2007 Nov [cited 2020 Jun 13];60(5). Available from: https://pubmed.ncbi.nlm.nih.gov/17872917/

7. Yang JM, Chen YF, Tu YY, Yen KR, Yang YL. Combinatorial Computational Approaches to Identify Tetracycline Derivatives as Flavivirus Inhibitors. PLoS One [Internet]. 2007 May 9 [cited 2020 May 25];2(5). Available from: https://pubmed.ncbi.nlm.nih.gov/17502914/

8. Castro JEZ, Vado-Solis I, Perez-Osorio C, Fredeking TM. Modulation of Cytokine and Cytokine Receptor/Antagonist by Treatment with Doxycycline and Tetracycline in Patients with Dengue Fever. Clin Dev Immunol [Internet]. 2011 [cited 2020 May 25];2011. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3065216/

9. Kane SP, Phar, BCPS. Sample Size Calculator [Internet]. [cited 2020 13 July]. Available from: https://clincalc.com/stats/samplesize.aspx

10. Statistics. Statistics » COVID-19 Hospital Activity [Internet]. [cited 2020 Jul 10]. Available from: https://www.england.nhs.uk/statistics/statistical-work-areas/covid-19-hospital-activity/

11. Coronavirus (COVID-19) in the UK [Internet]. [cited 2020 Jul 12]. Available from: https://coronavirus.data.gov.uk/archive

12. Liang W-H, Guan W-J, Li C-C, Li Y-M, Liang H-R, Zhao Y, et al. Clinical characteristics and outcomes of hospitalized patients with COVID-19 treated in Hubei (epicentre) and outside Hubei (non-epicentre): a nationwide analysis of China. Eur Respir J [Internet]. 2020 Jun [cited 2020 Jul 9];55(6). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7144336/

13. New York [Internet]. The COVID Tracking Project. [cited 2020 Jul 13]. Available from: https://covidtracking.com/data/state/new-york

14. Suleyman G, Fadel RA, Malette KM, Hammond C, Abdulla H, Entz A, et al. Clinical Characteristics and Morbidity Associated With Coronavirus Disease 2019 in a Series of Patients in Metropolitan Detroit. JAMA network open [Internet]. 2020 Jun 1 [cited 2020 Jul 9];3(6). Available from: https://pubmed.ncbi.nlm.nih.gov/32543702/

15. La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, et al. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. Eur J Clin Microbiol Infect Dis. 2020 Apr 27;39(6):1059–61.

16. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting Infectious SARS-CoV-2 From Diagnostic Samples. Clin Infect Dis [Internet]. 2020 May 22 [cited 2020 Jun 24]; Available from: https://pubmed.ncbi.nlm.nih.gov/32442256/

17. Peyriere H, Makinson A, Marchandin H, Reynes J. Doxycycline in the management of sexually transmitted infections. J Antimicrob Chemother. 2018 Mar 1;73(3):553–63.

18. Marlin GE, Cheng S. Pharmacokinetics and Tolerability of a Single Oral 600-mg Dose of Doxycycline. Med J Aust [Internet]. 1979 Jun 16 [cited 2020 Jun 26];1(12). Available from: https://pubmed.ncbi.nlm.nih.gov/38382/

19. Jones R. Single 600-milligram Oral Dose of Doxycycline in the Treatment of Gonorrhoea. Med J Aust [Internet]. 1981 Feb 21 [cited 2020 Jun 26];1(4). Available from: https://pubmed.ncbi.nlm.nih.gov/7231283/

20. No title [Internet]. [cited 2020 Jun 26]. Available from: https://www.medsafe.govt.nz/Profs/Datasheet/d/Doxinetab.pdf

21. Yim CW, Flynn NM, Fitzgerald FT. Penetration of oral doxycycline into the cerebrospinal fluid of patients with latent or neurosyphilis. Antimicrob Agents Chemother. 1985 Aug;28(2):347.

22. Dotevall L, Hagberg L. Successful Oral Doxycycline Treatment of Lyme Disease-Associated Facial Palsy and Meningitis. Clin Infect Dis. 1999 Mar 1;28(3):569–74.