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Research Article

Outcome of Combination Therapy of Trimethoprim and Zinc for Treating Respiratory RNA Virus Infections: Influenza and SARS-CoV-2

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Abstract

Background: Despite extensive immunization programs, respiratory RNA viruses including influenza and coronavirus have been challenging man with epidemics and pandemics. Thus, there is a need for drug treatment of such viruses.

Methods: The study objective is to examine outcomes of using a combination therapy of trimethoprim and zinc (Tri-Z) for treating influenza and SARS-CoV-2 (COVID-19) virus infections. In a retrospective observational study, comparison was made between Tri-Z, Oseltamivir, and supportive (no antiviral) treatment for managing natural influenza infection. Data were analysed in 2020. Tri-Z was then used for treating SARS-CoV-2 patients, follow up was for 6 months. Records of patients with natural influenza were examined, if they had no other acute or chronic disease, not receiving medication and had no immunization within 12 months. Patients' age, sex, BMI, immune status, smoking status, time to presentation, and viral load were evaluated. Those with SARS-CoV-2 illness were compared to general population. Influenza patients choose between oral Tri-Z (200 mg trimethoprim and 30 mg zinc tablets, simultaneously, 8 hourly); Oseltamivir (75 mg, 12 hourly); or supportive treatment. SARS-CoV-2 patients were treated with Tri-Z; there was no other treatment available. Duration of illness, its severity (area under the curve), and time patients were unable to do their normal daily activities (off work), were evaluated from symptoms score cards. Time to negative upper away viral swabs was also examined.

Results: In the period from 2009 -2011, 90 patients had influenza A virus. They received Tri-Z, Oseltamivir or supportive treatment (n = 30 per treatment group). Medians (and ranges) of duration of their illness in days were 2 (1.5 – 3.5) for Tri-Z, vs. 4.5 (3 – 7.5) for Oseltamivir, and 5.5 (4.5 – 8.5) for no antiviral group; $p < 0.05$. Means (and 95 % confidence intervals) of severity of illness were 0.84 (0.83 – 0.85) for Tri-Z; vs 0.87 (0.85 - 0.89) for Oseltamivir, and 0.94 (0.917 – 0.963) for ST, $p < 0.05$. The sum of recorded days, when patients were unable to do their normal daily activities (off work), per group was 29.5 for Tri-Z vs. 77 for Oseltamivir and 105.5 in no antiviral group, $p < 0.05$. Viral swabs became negative in 2 (1-3.5) days with Tri-Z, vs. 3 (1 – 4) with Oseltamivir and 4 (2 – 7) in no antiviral, $p < 0.05$. In the year 2020, eleven patients with SARS-CoV-2 illness received Tri-Z. Their median (and rang) duration of illness were 3 (2-5) days, and that of positive viral swabs was 4 (3-5) days; except a patient with severe pneumonia, it was 21, and 8 days, respectively which is significantly ($p < 0.05$) lower than that for general population. No long-term sequelae or recurrences were reported during follow up.

Conclusion: Like Oseltamivir, Tri-Z reduced duration, severity of illness, time off work and shedding of influenza virus comparing to supportive (no antiviral) treatment. It reduced the duration of SARS-CoV-2 illness and duration of positive viral swabs comparing to general population. Therefore, Tri-Z treats respiratory RNA viruses such as influenza and SARS-CoV-2. Decision to treat is based on clinical judgement.

Keywords: Influenza virus; Coronavirus; Tri-Z; Trimethoprim; Zinc; RNA virus; COVID-19; SARS-CoV-2; Respiratory virus

Introduction

Synthesis of viral RNA and DNA requires polymerase enzymes.1 While DNA polymerases have read-proof abilities with correction facilities, RNA polymerases do not have such qualities by-which errors (mutations) are much easier to occur [1]. Some of these mutations allowed RNA virus to challenge man with devastating illnesses. RNA viruses such as influenza virus and coronavirus require intact host cell; membrane, nucleus and cytoplasmic cytosol; to infect and replicate. Enucleated cells were shown to resist influenza virus infection and to reduce the yield of coronavirus [2,3]. Directing therapy to interfere with the host cellular mechanisms appears to hinder RNA virus replication [4,5]. A combination therapy of trimethoprim and zinc (Tri-Z) was shown to treat respiratory RNA virus infections [5]. In this retrospective observational study, we examine the outcomes of using Tri-Z to treat seasonal influenza, comparing to those of Oseltamivir and supportive (no antiviral) treatment. The outcomes of treating patients with SARS-CoV-2 at home was also examined.

Methods

Retrospectively, cases that fulfilled the following criteria were examined. Patients were managed according to the National Institute of Clinical Excellence (NICE) and World Health Organization (WHO) guidelines and regulations. Informed consent was obtained for treatment, and for medical data to be, confidentially, included in studies. The retrospective nature of the study was discussed with the Health Research Authority, United Kingdom, who waived the need for ethics approval. For SARS-CoV-2, it was considered clinical need of patients. Diagnosis of viral infection was made from clinical presentation and identification of the virus from upper airway samples [6,7]. Influenza and SARS-CoV-2 viruses were identified using RT-PCR as previously described [8-11]. At least 3 folds increase in anti-hemagglutinin antibody titer, 4 weeks later, confirmed the diagnosis of influenza [6]. Absence of other identifiable pathogens confirmed SARS-CoV-2 infection [7]. Patients with influenza infection received either Oseltamivir, Tri-Z or no antiviral (supportive treatment). Whereas patients with SARS-CoV-2 received Tri-Z. Tri-Z was given at a dose of 200 mg trimethoprim and 30 mg zinc tablets, 8 hourly [5]. The dose of Oseltamivir was 75 mg, twice a day, as previously recommended [12]. Supportive treatment was in the form of paracetamol and no antiviral medication [6]. All medications were taken orally.

Patients were given paracetamol (500 mg/tablet) as required / 4 hours. Paracetamol was shown to have no effect on viral shedding, temperature or clinical symptoms in patients with PCR-confirmed influenza [13]. Patients were followed up for 6 months. Patients presented with fever (above 37.5 oC); plus at least one respiratory symptom; plus at least one constitutional symptom [12,14,15]. To avoid confounding factors that may affect outcome of influenza treatment, we looked for patients with records that did not have chronic illness, health condition or medications that may affect their immune system and they had no contraindication to the medications [16-18]. However, all patients with SARS-CoV-2 were included because they were compared to disease outcomes in general population [18,19]. Patients were monitored closely using symptoms score cards as previously described [20,21]. Each symptom was graded from 0 to 3 (0 for no symptom, 1 for mild, 2 for moderate and 3 for severe) for ease of recording [22]. Patient's inability to do their daily activities (off work) was also recorded. The patient's temperature was that of their tympanic membrane [23].

Overall symptoms score indicated severity of illness at presentation [24]. Time from onset to presentation, as it effects duration of illness, was determined [25]. Duration of illness was calculated from presentation (time 0) to the time when all symptoms were alleviated, patients were able to do normal daily activities and without recorded recurrence within 4 weeks later [26-28]. Symptom sore less than or equal 1 and remained for at least 24 hours was indicated start of disease alleviation[24]. Full blood counts and C-reactive protein were measured to determine disease activity and to differentiate between bacterial and viral infections [6,7,29]. Serum zinc, copper, and iron levels were measured to ensure that there were no deficiencies [30,31]. Serum anti-hemagglutinin and anti-neuraminidase antibody titres were checked at presentation to determine patient immune status to the illness, as previously described [32-34]. Diagnosis was confirmed, four weeks later, by measuring at least 4 fold increase in anti-hemagglutinin antibody titres [6]. Differential diagnosis to exclusion of bacterial infection was made clinically and from full blood count and sputum culture [35]. The status of viral shedding and viral load at presentation were determined from upper airway swabs (nose and throat swabs for influenza virus and nasopharyngeal swabs for SARS-CoV-2) [6,7]. Swabs were taken at presentation and daily until negative. Duration of viral shedding was defined as the time from presentation to the time of first negative swab with two subsequent negative swabs.

Statistics

Statistical analysis was carried out using SPSS subscription software on Apple Mac platform. Data for age, sex, body mass index, smoking status and blood test results, were tested for normality of distribution. Then, parametric student t test or non-parametric Mann-Whitney tests were applied to determine significance of difference between treatment groups [36]. Data for sex and smoking status were analyzed using ANOVA [37]. We applied repeated analysis of variance to determine the significance of change in symptoms scores with time in each treatment group.37

To evaluate the significance of difference in change in symptoms scores between groups Wilcoxon signed rank test was applied [38]. The area under the curve (AUC) for each patient symptom scores was measured using trapezoids rule [39]. Then we compared the groups for significance of difference by applying non-parametric test, Mann-Whitney. Data were presented at the means and 95% confidence.

The significance of difference between groups in the duration of fever and days off work were examined at the median using Wilcoxon singed rank test and distribution by Friedman's Tow-Way analysis of variance, significance level was set at 0.05 [40]. Based on previous observation that a clinically significant difference in the median time to alleviation of symptoms of 5 days occurred in patients who were febrile at entry, it was calculated that 30 patients per group would be needed [5]. The probability of obtaining an earlier time of alleviation with Tri-Z or Oseltamivir compared with no treatment was estimated as 60%. A study with 75 patients (25 per group) was estimated to have a power of more than 90% for a two-tailed test at the 5% level of significance [41]. Therefore, including 30 patient per group ensure power of the study exceeds 90%.

For SARS-CoV-2, sample size calculation was based on previously reported population mean of duration of treatment to recovery or death of 18.1 days and estimated standard deviation of 3 days [42]. Based on findings from influenza patients, setting the null value at 14 days, power of 90% and significance of 0.05; then applying 2-ways one sample T-Test using SPSS subscription program, 8 patients were required[43]. Therefore, including 11 patients, ensures that power of the study exceeds 90%.

Key Points

a) Question: What are the outcomes of using a combination of trimethoprim and zinc (Tri-Z) for treat respiratory RNA virus such as influenza and SARS-CoV-2 (COVID-19)?

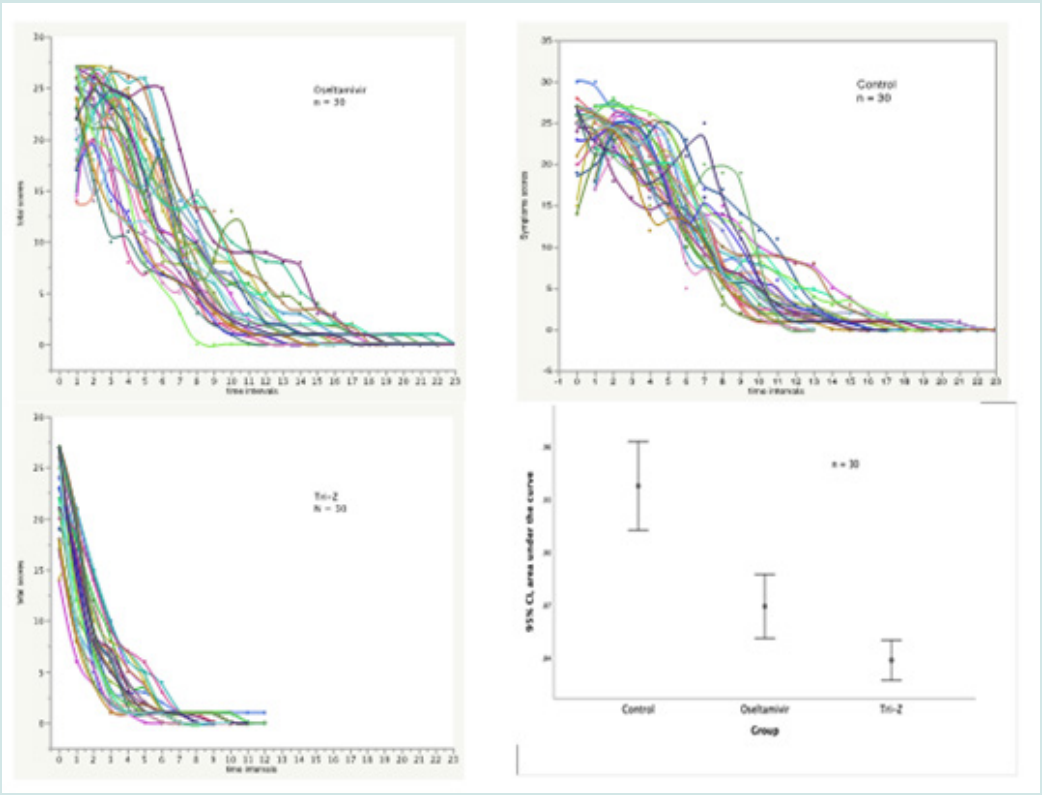
Findings: In patients with natural influenza, Tri-Z reduced duration of illness, its severity and viral shedding like Oseltamivir comparing to supportive (no antiviral) treatment. It had similar effects for treating SARS-CoV-2 compared to general population.

b) Meaning: Using combination of trimethoprim and zinc for treating respiratory RNA virus such as influenza and SARS-CoV-2 is warranted in as much as it shortens the illness, ameliorates its severity and reduces infectivity.

Results

Influenza

In the period from 2009 to 2011, we found 90 patients (63 males and 27 females) who were treated for influenza A virus infection and fulfilled the criteria for analysis. Their ages were 18.5 to 71 years. Their swabs were positive for influenza A virus. They received supportive treatment (no antiviral), Oseltamivir, or combination therapy of trimethoprim and zinc tables (Tri-Z), n = 30 patients per group. They were not taking any regular medication; had no underlying illness and they did not have influenza vaccination for the last 12 months. Patients ages, sex, BMI, smoking status, time from onset to presentation, serum zinc, iron, copper, leucocytes, neutrophil, and lymphocyte counts were similar between the groups. There was no recorded evidence of bacterial infections in all patients at the time of presentation. Patients' symptoms scores over time are shown in Figure 1. At presentation, symptoms scores and viral loads were similar between groups, Figure 2. Changes in scores over time were higher in Tri-Z group compared to Oseltamivir and supportive treatment groups, $p < 0.05$. That brought an earlier end of illness in the Tri-Z group than that with other treatment modalities. Reduction in symptoms scores with Oseltamivir were higher than that with supportive (no antiviral) treatment ($p < 0.05$). The means and 95 % confidence intervals (CI) of AUC were 0.84 (0.83 – 0.85)) with Tri-Z; 0.87 (0.85 - 0.89) for Oseltamivir, and 0.94 (0.917 – 0.963) with no antiviral treatment, $p < 0.05$, Figure 1. The duration of illness ranged between 1.5 – 3.5 days, median of 2 days for Tri-Z and 3 – 7.5, median of 4.5 days for Oseltamivir, and 4.5 to 8.5 days, median 5.5 days without antiviral treatment, $p < 0.05$. Number of days that patients were off work ranged between 2 – 5 days, (median 3.5 days, total 105.5 days) without antiviral; 0 – 2.5 days (median 1 days, total 29.5 days) with Tri-Z and 0 – 5 days (median 2.5 days, total 77 days) with Oseltamivir, $p < 0.05$. Duration of fever ranged between 1 – 3.5 days, (median 2.5 days, total 71 days) without antiviral, 0.5 – 2 days (median 1 day, total 26.5 days) with Tri-Z and 0.5 – 3.5 days (median 1.5 days, total 47 days) with Oseltamivir, $p < 0.05$. Paracetamol usage was similar between groups.



Graph showing patients symptoms scores against time periods. The interval between each time period is 12 hours. The left upper panel showing the scores from patients who received oseltamivir and the left lower graph showing that from patients treated with Tri-Z. The right upper graph shows changes in symptoms scores in the supportive treatment (referred to as control) group. The right lower graph shows comparison between the area under the curves in the groups. Data were plotted at the mean and 95 % confidence intervals (CI). Tri-Z = trimethoprim 200 mg and zinc oxide 36 mg.

Figure 1: Influenza patients' symptom scores.

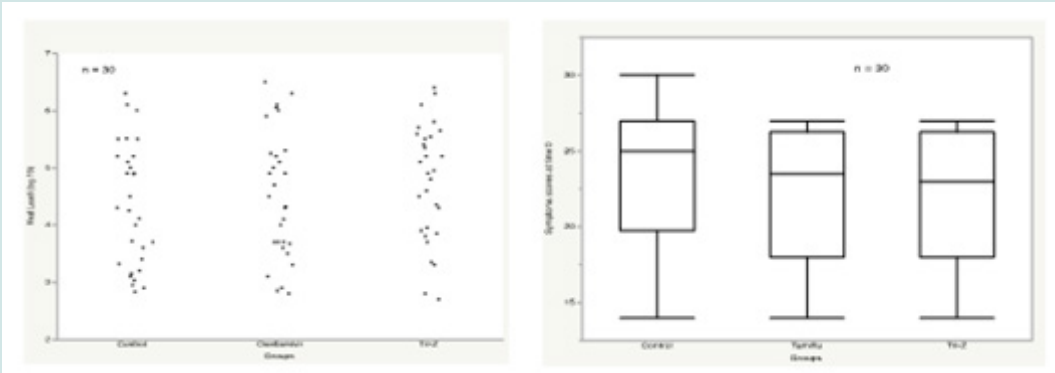
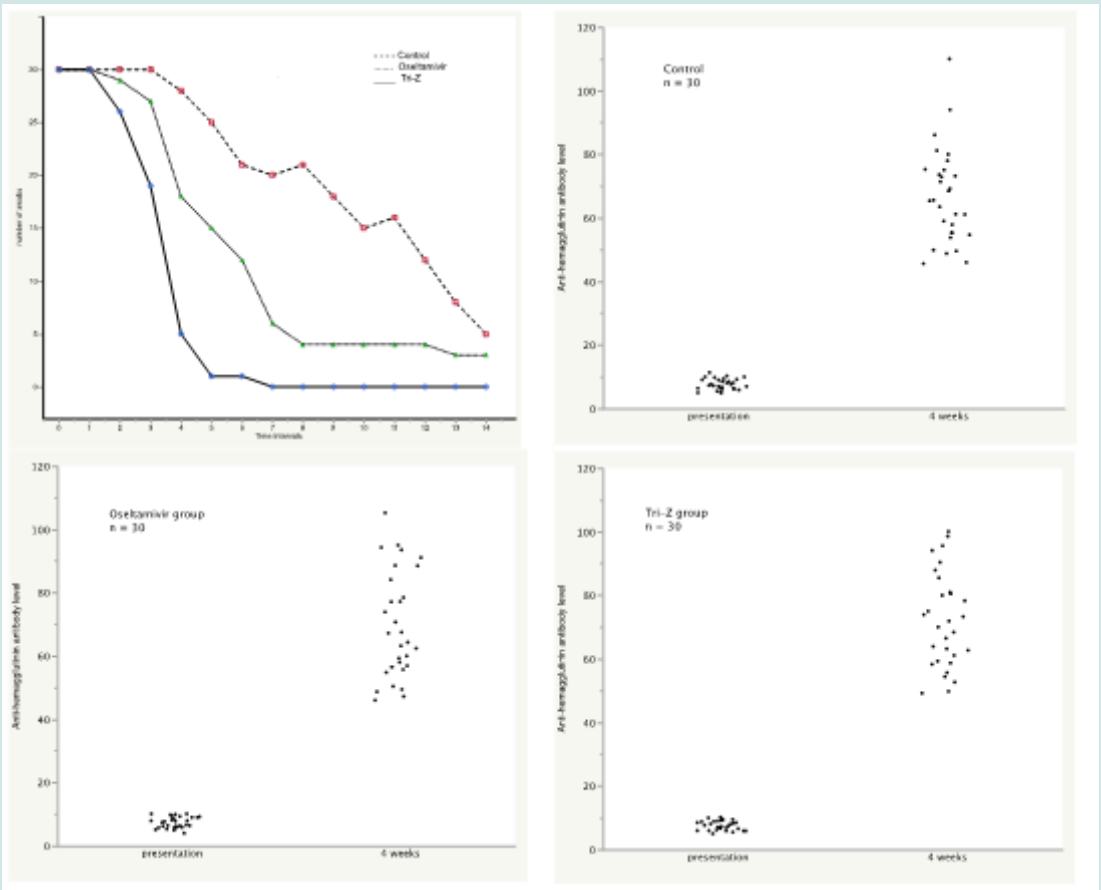


Figure 2 At presentation the viral load (log 10 copies / ml), left graph, and symptoms scores, right graph, were similar between the groups. Supportive treatment was referred to as control.

Figure 2: Influenza patients viral load and symptoms scores at presentation.

Side effects reported in Tri-Z group were change of taste (2 patients), and nausea (5 patients). Side effects in Oseltamivir group were abdominal pain (5 patients), vomiting (5 patients), nausea (4 patients), dyspepsia (4 patients), and rash (1 patient). In the control group nausea occurred in 5 patients, change of taste in 4 patients and sinusitis in 3 patients. Changes in numbers of positive swabs over time in each group are shown in Figure 3. Two days after treatment the swabs were positive in 28 patients with supportive treatment (93 %), 5 patients with Tri-Z (16.7 %) and 18 patients with Oseltamivir (60 %), $p < 0.05$. The duration of positive

swabs ranged between 1-3.5 days with Tri-Z. It was 1 – 4 days in 26 patients in Oseltamivir group, one patient had negative swabs by 6.5 days and 3 patients had positive swabs by end of one week after presentation. Without antiviral treatment, the duration of positive swab was 2 – 7 days in 25 patients, 5 patients remained to have positive swabs by end of one week after presentation. All swabs became negative by 2 weeks after presentation. At presentation, anti-neuraminidase antibodies were not detectable in all patients. Anti-hemagglutinin antibodies levels at presentation and 4 weeks later are shown in Figure 3. The increase in the anti-hemagglutinin antibody level was similar between groups.



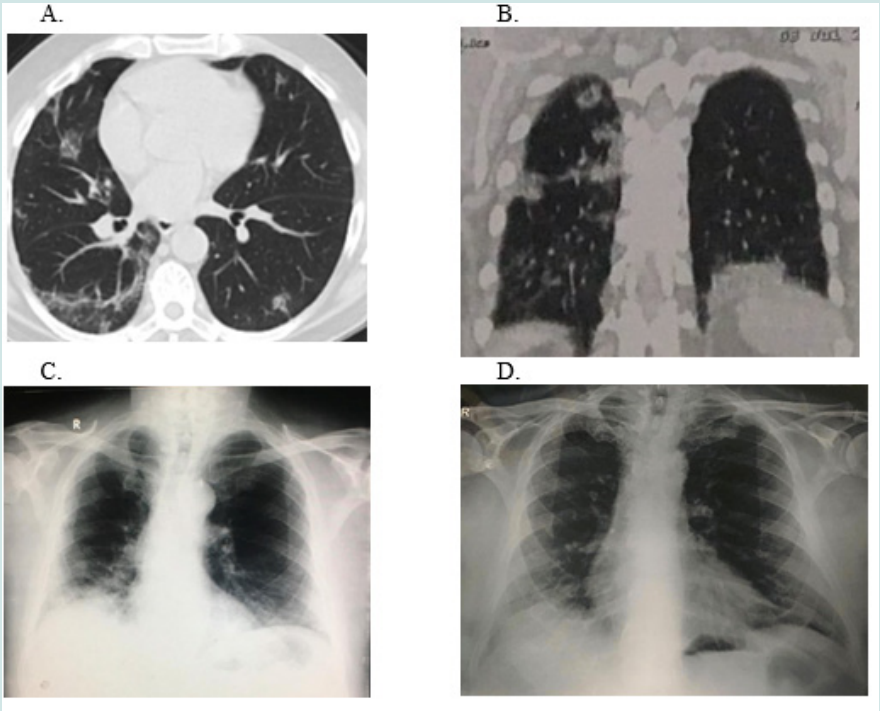
The left upper graph shows changes in the number of positive swabs for influenza virus over a period of 7 days. There was rapid reduction of positive swabs in Tri-Z group compared to Oseltamivir and supportive treatment (referred to as control) groups. Also, Oseltamivir reduced the number of positive swabs compared to controls. ANOVA test was applied and $p < 0.05$. The left lower, right upper and lower graphs show the anti-hemagglutinin levels at presentation and 4 weeks later. The marked increase in the level confirmed the diagnosis. The levels were similar between the groups. Oseltamivir and Tri-Z did not affect development of an immune response to the virus.

Figure 3: Changes in influenza patients' positive swabs and haemagglutinin antibody titters.

SARS-CoV-2

During the year 2020 of COVID-19 pandemic, 11 patients were treated using Tri-Z. Their ages ranged between 29 – 73 years, 2 females and 9 males. They had positive nasopharyngeal swabs for SARS-CoV2. Their symptoms were fever (11 patients), headache (11 patients), cough (10 patients), tiredness (10 patients), breathless (2 patients), loss of smell (2 patients), abdominal pain (1 patient). The interval between onset of symptoms and start of treatment ranged between 1-2 days except in one case (case 8) cough for 1 week prior to presentation. He had pneumonia and required 10 days of treatment, Figure 4. Another patient (case 9) had diabetes and hypothyroidism; aged 69 years old. Patients self-isolated and were treated at home. White cell counts and CRP results at presentation. Their serum zinc, copper and iron levels were within normal range for age. Duration of treatment was 3 to 5 days, except in case 8 it was 10 days. Duration of treatment were less than that for general population, $p < 0.05$. Duration of illness (time to resolution of symptoms and return to normal daily activities) was 2 to 5 days with median of 3 days, except case 8 it was 21. Duration of fever

was 1 to 3 days median of 1 day except case 8 it was 5 days. Time to negative swabs (viral shedding) was 3 to 5, median 4, days, except in case 8, it was 8 days. All patients fully recovered. No side effects were reported for Tri-Z. Follow up, 6 to 10 months did not show any recurrences and patients continued with their normal life activities. There was no long term coronavirus illness reported.



Case 2, it shows CT scan images A, B and X-ray C, before treatment. D. X-ray image 10 days after treatment.
Figure 4: Radiographs of COVI-19 patient.

Discussion

This retrospective evaluation shows that combined use of trimethoprim and zinc oxide (Tri-Z), like Oseltamivir, is effective in treating influenza virus infection in man. Both treatments reduced duration and severity of illness compared to supportive treatment. Time to recovery and areas under the curves of symptoms scores for both treatment groups were significantly less than that without antiviral treatment. Tri-Z appears to further reduce duration of illness compared to Oseltamivir. Factors that may influence this outcome such as viral load at presentation, duration of illness prior to starting treatment, patients' demographics and leukocyte counts were similar between treatment modalities. Anti-neuraminidase antibodies were also negative in all patients. Despite suppressing the illness, both Oseltamivir and Tri-Z allowed development of immune response. The levels of anti-haemagglutinins antibodies increased and were similar to that of patients who did not receive antiviral treatment at 4 weeks after infection. The apparent difference between Oseltamivir and Tri-Z may be related to mechanisms of action. Tri-Z was proposed to interfere with viral dependent host cellular mechanisms whereas Oseltamivir acts directly on the virus [5,44].

This may be further explained by understanding how RNA virus is cleared from its host combined with mechanism of action. Oseltamivir is a prodrug that is metabolised into active form "Oseltamivir carboxylate" [44]. It inhibits viral neuraminidase that is essential for viral replication and exit from the cell [45]. Thus, its effect is mainly intracellular. Therefore, clearance of the virus may require cell turnover. Tri-Z was shown to inhibits viral entry into the cells by inhibiting it from biding to its hemagglutinin cell receptor [5]. Thus, the virus can be cleared with mucus which is faster than cells turnover. [47-49] This may also explain why patients who received Tri-Z had shorter time to stop viral shedding compared to the other treatments. With deeper infection such as pneumonia, viral shedding is expected to take longer time as the virus is impeded into lower airway cells [50,51]. Alveolar cells and macrophages were reported to have slower turnover than that of cells in bronchi and bronchioles [48]. Patient with SARS-CoV-2 pneumonia had longer time to have negative swabs.

Coronavirus does not have neuraminidase activity [52,53] which may account for ineffectiveness of Oseltamivir in treating SARS-CoV-2 [54]. With Tri-Z, patients had earlier recovery from illness, return to normal daily activities and negative viral swabs

than that for general population [55,56]. Effectiveness of Tri-Z in coronavirus infection may be related to the virus relying on its host cellular mechanisms to attach, enter the cell and replicate [57]. It requires cysteine rich domain to be able to fuse with cell membrane whereby trimethoprim (TMP, part of Tri-Z) metabolites can adduct to such protein [58,59]. The virus needs cell surface receptors to attach and intact nucleus to replicate, thereby TMP may further imped the virus [60-63]. Zinc ions were reported to inhibit coronavirus invitro cell culture [64]. TMP and zinc separately did not show clinical effect on respiratory RNA virus infection.5 However, at a combination of TMP and zinc (Tri-Z) appears to act as dynamic molecule with wider mechanism of action, perhaps similar to a zinc figure [5,65-67].

It seems that Tri-Z and Oseltamivir may reduce duration of infectivity of patients. The duration of influenza viral RNA detection in upper airway samples (positive swabs) were shorter than that without antiviral treatment. Absence of viral RNA in respiratory samples using RT-PCR indicates absence of infectious virus in these samples [68]. Compared to Oseltamivir, Tri-Z brought about further reduction in duration of viral shedding which was associated with earlier termination of fever. Previous studies showed a relationship between duration of fever and that of viral shedding [69]. Some patients with Oseltamivir and those with supportive treatment continued to shed virus RNA for some days after recovering from the illness. This phenomenon was reported in asymptomatic influenza carriers [70]. It shows that infectivity of treated patients may continue after cure of illness. Although the virus primarily infected the respiratory system in our patients, it may be transmitted via another route such as stool [71].

Part of the socioeconomic impact of respiratory RNA virus infection is inability to carry out normal daily activities (off work). While Tri-Z and Oseltamivir reduced the number of days patients were off work compared to supportive treatment, other factors might have affected this outcome [72,73]. Oseltamivir group had larger variation than that for Tri-Z group which may be related to longer duration of illness and side effects. There were more side effects with Oseltamivir than with Tri-Z. These studies have limitations. It is a retrospective observation of small samples. Although there was a group of patients who received only supportive (no antiviral) treatment which may act as control, it does not replace role of a placebo. However, findings from the study may act as a background to develop a randomized placebo-controlled trial.

Conclusions

Like Oseltamivir, using a combination of trimethoprim and zinc oxide at specific ratio (Tri-Z) can hinder respiratory RNA virus infection and replication in as much as it can treat influenza virus and coronavirus infections. It shortens duration of illness, severity, and viral shedding in upper airway samples. Furthermore, it may ameliorate the economic impact of illness by decreasing the time taken off work. Decision to treat is dependent on clinical judgement.

Declaration of Interest

There is no conflict of interest to declare.

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