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Pharmacogenomics to Drive COVID-19 Therapy for Best Outcome in a Low Resource Setting

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ABSTRACT

Corona virus disease 2019 (COVID-19) has taken the world by storm with global infectivity and mortality of 3.5%. Since there is no specific treatment for COVID-19, several drugs have been repurposed to combat infection, these include drugs like anti-malarial – chloroquine, hydroxychloroquine, anti- diarrheal– loperamide and antipsychotic-promazine, which have been considered to be effective inhibitors as of viral binding to ACE2 receptor. The administration of these drugs is currently random and is the key factors responsible for varied treatment response, hence genes involved in drug metabolism should be analysed before planning therapy. Genes involved in metabolism of the listed drugs are ABCB1, CYP1A2, CYP2C8, CYP2C19, CYP3A4 and CYP2D6. Unpublished pharmacogenomic data from our internal cohort (75 cases) was analyzed to predict likely-responders and non-responders to propose drugs for COVID-19 drug therapy in our population. Preliminary data from random individuals without bias indicates that both anti-malarials at standard dose will benefit 98% of our cases (in absence of co-morbidities), while 11-85% of individuals would require dose reduction/alternatives for loperamide and promazine. Anti-malarials like chloroquine, hydroxychroloquine can be prescribed for prophylaxis and as first line of therapy in absence of comorbidities. Simple genotype testing of ABCB1, CYP1A2, CYP2C19 and CYP2D6 is an indispensable tool to predict treatment outcomes of loperamide and promazine for COVID-19 patients.

Keywords - COVID-19 pandemic, drug-repurposing, pharmacogenomics

1. Introduction

The current Corona virus disease 2019 (COVID-19) has taken the world by storm. A pandemic which originated in Wuhan, China is now a global threat to large population. The health statistics is going haywire and the active cases are being reported at an alarming rate (about 64,80,148 – 17th August 2020) [1] From the universal trend, the mortality rate across populations from COVID-19 is ranging from 1-3% in Australia,

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Russia, India and USA to 4-9% in Spain, Germany and Iran. China has reported about 93% of recovered cases [1]. However, treatment protocols have not been discussed in each case of mortality or recovery but it involves targeting the infectivity of the virus, interfering with its lifecycle or addressing its effects on upper respiratory tract and GI tract [2]. The traditional way of evaluating a potential benefit of therapy is to assess a chemical by in vitro experiments, test it on animal models and subsequently pass the compound through multiple clinical trials in humans. All this is not feasible in the current scenario; hence drug re-purposing is one of the best options based on previous experience [3]. The treatment protocol up till now in treating the COVID-19 has been based on clinical acumen and availability of the drugs. There are no specific therapeutics approved by the Food and Drug Administration (FDA) to treat people with COVID-19. Factors like drug-drug interaction, drug dosage and time frame of treatment has not been worked out in the light of uncertainty. Other drugs including steroids, Dexa and Methylprednisone and IL6 monoclonals are being used for halting the progression of disease in hospitalised patients (Oxford protocol). The follow-up is also based on reduction of symptom severity or mortality [4]. The WHO SOLIDARITY protocol has proposed four drug mega trial, which includes an experimental antiviral compound called remdesivir; antimalarials - chloroquine and hydroxychloroquine; a combination of two HIV drugs - lopinavir and ritonavir are available immunomodulator [3]. Another emerging drug molecule from in vitro studies is the anti-diarrheal opiod receptor agonist, loperamide [5]. Based on the recent report of Zhang and Liu (2020), chloroquine, emodin, and promazine, apart from the monoclonal antibody, scFv80R, could be used as alternatives for the treatment of COVID-19 [6]. Antiretroviral drugs have also been administered individually and in combinations often masking the intra drug interactions and relying on the protocol based trial and error approach, whereas the monoclonal based biologicals are expensive and not easily accessible [7- 9]. They potential use of health supplements like Vitamin A, Nicotianamine, zinc that can boost the immune system have also been discussed [6]. There are few medicinal plants with antiviral properties also being proposed for therapeutics [10]. The first line of treatment for a low resource country like India should be the use of one of the three types of drugs from the suggested list that is anti-malarial, anti-diarrheal and anti-psychotics. These drugs are relatively inexpensive, easy to access and their efficacy, side effects are well documented.

The mechanism of action of all the three type of drugs is to inhibit the Angiotensin converting enzyme 2 (ACE2), a functional receptor for the coronavirus (CoV) that causes severe acute respiratory syndrome (SARS) and is also the receptor for the SARS-CoV-2 responsible for COVID-19 [6]. ACE2 mediates the entry of the virus into the cell after binding with spike S protein present on the viral surface. Since this is the sole receptor for the entry of SARS-CoV-2, blocking ACE 2 using inhibitors is important for the prevention /treatment of the infection [11,12]. Though age and co-morbidities are recurrently being cited as the cause of severity and progression of COVID-19 to mortality, the genetic make-up and role of ethnic polymorphisms in devising the most appropriate therapy has not been considered [13]. In this paper we wish to highlight the importance of employing pharmacogenomic/companion diagnostic approach to identify patient genotypes to select the most appropriate drug therapy and choose one of the most suitable compounds for preventing infectivity in our population. This genotype based evaluation of drug response can be beneficial for reducing infection, morbidity and mortality caused by COVID-19, as well as, save medical resources for individuals susceptible for the disease. Relevant pharmacodynamic and pharmacogenomic information regarding these three types of drugs is given in Table 1.

Table 1 - Some drugs useful in treating COVID-19 and the genes involved in their pharmacogenomics

DRUG	GENERIC NAME	USED AS	SUBSTRATE
Chloroquine	Chloroquine phosphate	Anti-Malarial	CYP2C8, CYP3A4, CYP2D6
Hydroxychloroquine		Anti-Malarial	CYP3A4, CYP2D6
Imodium	Loperamide	Anti-Diarrheal	CYP2C8, CYP34A, ABCB1
Promazine	Promazine	Antipsychotic	CYP1A2, CYP3A4, CYP2C19

1.1 Anti-Malarial

Chloroquine phosphate is an antimalarial drug that has been repurposed for the treatment of extra intestinal amoebiasis, HIV, auto immune diseases like SLE and rheumatoid arthritis [14]. It has been proposed to be one of the first drugs to combat the COVID-19 infection with several clinical trials with positive preliminary outcomes [15] Absorption after oral ingestion is completely gastrointestinal (90%), and the volume of distribution is large owing to its extensive tissue sequestration, particularly in the liver, spleen, kidneys and erythrocytes [16]. Chloroquine is metabolized by the liver by the enzyme CYP2C8 to its active metabolite desethylchloroquine, 50% of which is cleared by the kidneys unchanged. Thus, the dose needs to be altered for individuals with abnormal renal function [16]. If chloroquine hydrochloride is given intravenously, it must be administered by slow, constant infusion to avoid respiratory depression, hypotension, heart block, cardiac arrest and seizures that may occur with transient toxic levels. A dose of 300 mg base q8–12h may also be given by intramuscular injection. Two common, naturally occurring polymorphic variants of CYP2C8 are CYP2C8*2 and CYP2C8*3 which display altered drug metabolism compared to the reference allele CYP2C8*1. Both alleles code for enzymes with decreased performance [17]. Other secondary enzyme involved in the metabolism of this drug is CYP3A4, however, the affinity for chloroquine is highest for CYP2C8 [18] group at the end of a side chain and is manufactured as Hydroxychloroquine sulfate (Plaquenil). Along with chloroquine, it is postulated to be a potential treatment for the COVID-19 [19, 20]. An in vitro study found that Hydroxychloroquine is more potent than chloroquine for inhibiting SARS-CoV-2 [21]. It is N-dealkylated by CYP3A4 enzyme to the active major metabolite desethylhydroxychloroquine, as well as the inactive metabolites desethylchloroquine and bidesethylchloroquine [22]. Both chloroquine and hydroxychloroquine inhibit the CYD2D6 enzyme after utilizing it as a substrate in a feedback mechanism [23]. CYP2D6 is required for metabolising of 20-25% of drugs [24] and hence, genotyping of CYP2D6 is essential along with CYP2C8 before administering chloroquine or hydroxychloroquine especially when planning a prescription with other drugs.

1.2. Anti-Diarrheals

Loperamide, commonly known as Imodium, is usually available as a hydrochloride and is effective against diarrhea resulting from gastroenteritis or inflammatory bowel disease. It is an opioid receptor agonist and acts on the mu opioid receptors in the myenteric plexus of the large intestines; it does not affect the central nervous system like other opioids [25]. Loperamide was able to inhibit the replication of MERS-CoV, SARS-CoV, as well as HCoV-229E in the low micromolar range, which suggests that it may have a broad spectral anti-viral activity [26]. Loperamide is almost completely extracted by the liver, where it is predominantly metabolized, conjugated and excreted via the bile. Oxidative N-demethylation is the main metabolic pathway for loperamide metabolism, which is mediated mainly through CYP3A4 and CYP2C8 enzymes. Due to this very high first pass effect, plasma concentrations of unchanged drug remain extremely low. [26] The protein encoded by the ABCB1 gene (also called P- glycoprotein) is an ATP-dependent drug

efflux pump for xenobiotic compounds with broad substrate specificity and the ABCB1 polymorphism (*2) G3435T affects its plasma levels with ABCB1 TT3435 carriers having high loperamide plasma levels [27].

1.3. Anti-Psychotics

Promazine is a phenothiazine derivative that has a role as a dopaminergic antagonist, a H1- receptor antagonist, a muscarinic antagonist, a serotonergic antagonist that is used as antipsychotic drug and an antiemetic drug [28]. Promazine has been found to exhibit significant effects by inhibiting the replication of SARS-CoV through blocking the interaction of S protein and ACE2 [29]. Promazine is metabolized in liver primarily to N-desmethylpromazine and promazine sulfoxide. Enzymes CYP1A2 and CYP3A4 are the main isoforms responsible for the 5-sulphoxidation, while CYP1A2 and CYP2C19 are the basic isoforms that catalyze N- demethylation of promazine in human liver. CYP1A2 is the most important isoform for this drug [30].

2. Materials and methods

We have performed a literature survey for the available options for reducing COVID-19 infections and found that anti-malarial, anti-diarrheal and anti- psychotic drugs that inhibit the action of ACE 2 by either blocking it or preventing its interaction with the virus are most suitable for treating patients to prevent infections. The suitability of the 3 drugs discussed in this study is because of their low cost and availability. The pharmacogenomics and chemical composition of the above drugs was assessed by using the National Centre for Biotechnology Information - PubChem Database. The genes relevant for the identified drugs involved in absorption/transportation and metabolism were ABCB1, CYP1A2, CYP2C8, CYP2C19 and CYP3A4. Polymorphisms in these genes need to be evaluated before we can comment about the most appropriate agents for COVID-19 treatment in Indian population (Table 2).

Pharmacogenomics and companion diagnostics are routinely offered in the Department of Genetics & Molecular Medicine, Kamineni Hospitals. The test is offered to patients referred from different specialties for conditions like cardiovascular, rheumatism, infectious diseases, chronic renal disorders, psychiatry and oncology. Since sequences in COVID-19 infected\ individuals are yet to be carried out, we have looked for naturally occurring polymorphisms in this cohort of random group of individuals to establish variations in genes predicted to be crucial to play a role in COVID-19 therapy. The number of individuals that metabolize different drugs at different rates in our population were assessed and categorized as Hyper, Ultra Rapid, Normal, Intermediate and Poor metabolizers [16,17,25,30] based on 20 genes and 68 single nucleotide Polymorphisms In the present study we have assessed the allele frequencies and genotypes of the four candidate gene polymorphisms from the data available with us from 75 random individuals and classified them as likely responders/non responders for the drugs proposed to treat COVID-19. These were also compared with global frequencies from available databases.

3. Results

We have identified individuals as normal, poor and extensive transporters and metabolizers based on the genotyping of ABCB1, CYP1A2, CYP2C19, CYP3A4, CYP2C8 and CYP2D6. The variant allelic frequencies identified in our samples matches the global data especially South Asian frequencies except CYP2C19 *17 frequency, which is high in our cohort (Table 2). Based on our data (Table 3), we found that, about 52% of individuals have reduced activity of CYP2D6 indicating drug and dose adjustment has to be done before prescribing chloroquine and hydroxychloroquine. Similarly, 37.97% of individuals were poor

transporters, while 44.73% had reduced function and 15.78% cases had normal function of ABCB1 gene, indicating that loperamide treatment with standard recommended dose will benefit only 16% of individuals

Table 2: Genes, relevant functional polymorphisms and their frequency important for three types of drugs (anti-malarial, antidiarrheals and anti-Psychotics which act through ACE2 receptor to prevent Covid19 infection)

GENE	ALLELES	MARKER	TOPMED	1000 G Global	1000 G South Asian	Present Cohort (N=75)
ABCB1	*2	rs1045642	0.59	0.6	0.42	0.61
CYP1A2	*1C	rs2069514	0.13	0.2	0.08	0.07
CYP1A2	*1F	rs762551	0.67	0.62	0.53	0.51
CYP2C8	*2	rs11572103	0.05	0.05	0.01	0.02
CYP2C8	*3	rs10509681	NA	0.04	0.03	0.03
CYP2C19	*2	rs4244285	0.16	0.22	0.35	0.34
CYP2C19	*17	rs12248560	0.19	0.15	0.13	0.23
CYP3A4	*22	rs35599367	0.03	0.015	0.00	0.006
CYP2D6	*2	rs16947 and rs1135840	0.37 NA	0.36 0.59	0.2 0	0.102
CYP2D6	*4	rs3892097	0.14	0.09	0.109	0.075
CYP2D6	*6	rs5030655	0.007	0.0048	0.001	0.006
CYP2D6	*7	rs5030867	0.0003	0.0018	0.009	0.006
CYP2D6	*9	rs5030656	0.017	0.0072	0	0
CYP2D6	*12	rs5030862	0.0003	Not there	Not there	0.013
CYP2D6	*17	rs28371706	Not there	0.0591	0	0.006
CYP2D6	*20	rs72549354	None	None	None	0.082
CYP2D6	*36	CYP2D7/2D6				0.034
CYP2D6	*41	rs28371725	0.071	0.063	0.122	0.047

Table 3: Percentages of individuals with normal and varied metabolism based on genotyping from individuals in our cohort

S.No.	GENE	NORMAL	INTERMEDIATE	POOR	HYPERINDUCIBLE
1	ABCB1	15.78% (*1/*1)	44.73% (*1/*2)	37.97% (*2/*2)	
2	CYP1A2	65.3 % (*1A/*1A), (*1A/*1F)	1.3% (*1A/*1C)		33.4% (*1F/*1F) (*1C/*1F)
3	CYP2C8	95% (*1/*1)	5% (*1/*2) (*1/*3)		
4	CYP2C19	18% (*1/*1)	43% (*1/*2, *17/*2)	12% (*2/*2)	
5	CYP3A4	98.3 % (*1/*1)	1.3% (*1/*22)		
6	CYP2D6	43.83% (*1/*1, *1/*2, *1/*39, *10/*2, *2/*10, *2A/*10, *34/*10, *34/*2A, *34/*34, *39/*2A, *39/*34, *39/*39)	23.28% (*1/*10, *1/*36, *1/*41, *10/*17, *1/*36, *2/*4, *2A/*7, *39/*41, *39/*4A, *4/*10)	28.76% (*1/*4, *10/*4A, *2A/*2A, *20/*20, *36/*36, *36/*41, *41/*12, *4A/*4A, *6C/*11, *10/*4A, *11/*11)	ULTRA RAPID 4.10% (*1/*1 +gene duplication, Gene duplication, *1/*4A or *10/*4M gene duplication)

CYP1A2 polymorphisms indicated that 65 3% of our patients had *1/*1 allele and were normal metabolizers, while 1.3% showed reduced activity and 33.4% harboured hyper inducible allele *C or *F, implying that caution is required if multiple drugs are being co-administered. Individuals with *C and *F are also prone to adverse effects with caffeine and nicotine metabolism, which are common lifestyle factors. Hence, promazine treatment will show adverse reaction especially if given in combinations. On assessing the CYP2C19 polymorphisms, 18% had alleles responsible for normal metabolism, 43% for reduced and 12% for poor metabolism of promazine, based on this it can be estimated that 55% of our population cannot be given standard dose of promazine based on the functional polymorphisms of this gene. About 98.3% of the individuals were normal metabolizers of CYP3A4 based on its polymorphisms, while only 1.3% showed reduced activity. Hence majority of our cases can be given standard dose of all the four medications based on CYP3A4 polymorphism if other relevant gene polymorphisms are also indicative of normal metabolizers.

4. Discussion

COVID–19 has taken the centre stage of public health globally. Though specific approved virus specific treatment is currently unavailable, various drugs have been repurposed to alleviate infection and disease progression which is having an alarming toll on healthcare. The most cost-effective drugs for low resource settings like India are those which act as ACE2 inhibitors, which block this functional receptor of SARS CoV2, responsible for COVID-19. These include four common medicines Chloroquine, hydroxychloroquine, loperamide and promazine [6-7,20] It is well established that drug response is individual specific and based on genes involved in drug transport and metabolism. In the present study based on literature we identified drugs which maybe most relevant for treating COVID-19 in a low resource country like India until a specific treatment modality is globally approved. Preliminary data from our group of patients indicates that response to therapeutic outcomes can be predicted for Indian patients based on the pharmacogenomics. We have established that despite the small sample size, most of the functional polymorphisms have allele frequencies with global or South Asian databases, except for CYP2C19*17 which, this maybe unique to our population. Chloroquine and Hydroxychloroquine are antimalarials which primarily depend on activity of CYP2C8 and CYP3A4 for their metabolism and both of the drugs inhibit CYP2D6 activity. Chloroquine can be prescribed to individuals who are normal metabolizers for CYP2C8 (*1/*1) with standard dose, which suggests that 93-96% of the Indian population can safely be treated with the recommended drug dose of this drug if they are not any other medication, whereas reduced dosage has to be planned for individuals with *1/*2 or *1/*3 genotypes and an alternative drug is to be considered for individuals with two functionally ineffective alleles, like *2/*2, *2/*3 and *3/*3. Since CYP2D6 metabolises 20-25% of drugs in general, the prescription of chloroquine and hydroxychloroquine should be closely monitored especially in individuals with co-morbidities or multiple medication. Based on genotyping of CYP3A4 all three types of drugs can be prescribed to those with *1/*1 genotype at standard dosage, reduced dosage for individuals with one ineffective allele (*1/*2 or *1/*17) and alternatives for patients with two ineffective alleles.

Our data also indicates about 98% of individuals from our cohort are normal for CYP3A4 metabolism. However, 52% of them have reduced CYP2D6 activity and may have altered response to standard dose of other drugs they are using, if they are metabolized by CYP2D6. Loperamide depends for its absorption and transportation on ABCB1 genotype status and based on our samples only 15.78% of our patients would be suitable for standard dose of this drug while, 45% of ABCB1 *2 carries would require dose alteration and 38% would require alternatives. Promazine is metabolized by CYP1A2 and based on allele frequency in our

population it can be predicted to work efficiently at the standard dose for 65% of the individuals and with caution on co- administration of caffeine in 1% of the population, while 34% should be prescribed with alternatives to prevent adverse drug reactions especially if multiple drugs are being administered. Hence, the first line of preventative treatment for a low resource country like India based on this preliminary data should be the use of cost effective, easily available anti-malarial, anti-diarrheal and anti-psychotics drugs especially in individuals with no co-morbidities as the two genes. We propose that pharmacogenomic data useful to predict treatment outcome and a larger study is warranted for establishing pharmacogenomic based treatment protocols for our population. Polymorphism frequencies relevant for the metabolism of these drugs are favourable for the management of our population is 93-96% of CYP2C8 and 98% for CYP3A4 indicating antimalarials can be prescribed as prophylactic medicine to individuals as a first line in absence of co-morbidities. Usage of it has already been prescribed for asymptomatic healthcare workers by ICMR [31], with pharmacogenomic basis, we can adapt an evidence based practice and prescribe it for prophylaxis and therapeutic purposes in absence of co-morbidities and after genotyping to individuals who are already under prescription drugs.

5. Conclusion

Data available from our population indicates that CYP2C8 and CYP3A4 functional polymorphism are favourable for treating individuals with hydroxychloroquine, if they have no other co morbidities. Because 52% have CYP2D6 reduced activity and other drugs may not act efficiently. ABCB1, CYP2A1, CYP2C19 and CYP2D6 polymorphisms is an indispensable tool to predict treatment outcomes when treatment is planned with antimalarial, loperamide and promazine for COVID-19 patients.

6. Declarations

6.1 Study Limitations

The analysis was performed on a cohort to establish a pilot study and frequency of polymorphisms. Performing pharmacogenomic testing in COVID-19 affected individuals and then streamlining therapy is in future endeavour of this project.

6.2 Acknowledgements

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6.3 Funding source

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6.4 Competing Interests

The authors declare there are no competing interests.

6.5 Ethical Approval

Informed consent was taken from patients/parents/guardians prior to obtaining 2 ml of peripheral blood in EDTA vaccutainers or POC tissue as per the Institutional Ethics Committee of Kamineni Hospitals (Registration # ECR/ 58/ Inst/ AP/ 2013) guidelines. This study was carried out in accordance with the 4 recommendations of International Council of Harmonisation and Good Clinical Practice. All subjects/families gave written informed consent in accordance with the Declaration of Helsinki

6.6 Informed Consent

All patients were given pre-test counselling and written informed consent to use data for academic purpose was obtained prior to collecting blood sample.

Author Contribution

Gayatri R Iyer: Conceptualization, Data curation, Visualisation Writing – original draft. Syeda Zubeda: Methodology, Formal analysis. Aruna Priya Kamireddy: Data curation, Methodology. Qurratulain Hasan – Conceptualization, Supervision, Writing- review and editing All authors read and approved the final draft of manuscript

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