Original Article:
Chloroquine Phosphate Metabolism and Gender-based Phenotypic Analysis in Healthy Subjects’ Urine Following Oral Administration

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ABSTRACT

Background: Chloroquine, 4-N-(7-chloroquinolin-4-yl)-1-N,N-diethylpentane-1,4-diamine has promising activity against corona virus disease 2019 (COVID-19) and as such, it is imperative to thoroughly understand and determine the rate at which individual body systems metabolizes the drug. Chloroquine a known antimalarial drug belongs to the chemical class of 4-aminoquinolines.

Objectives: The study aimed to analyze Chloroquine and its metabolite in biological fluids of healthy subjects by simple thin layer chromatography (TLC), which is an efficient, and inexpensive method for quantifying Chloroquine and its metabolites.

Methods: A total of 30 healthy volunteers participated in the study by ingesting 500 mg of chloroquine, and the results were compared with side effects experienced by these subjects. Two brands of Chloroquine phosphate were used for the analysis and the urine were collected pre and post-drug administration and the intensities of the spots observed were compared with the reference standard stock solution. The same or greater intensity of sample spot indicates poor metabolizer, less intensity when compared to the stock spot indicates intermediate metabolizer while a much lesser intensity indicates an extensive metabolizer.

Results: There was a statistically significant difference between the brands of chloroquine used at P<0.05. 30% of the volunteers were assigned poor metabolizer phenotype, 50% were assigned extensive metabolizer phenotype, and 20% assigned Intermediate metabolizer phenotype based on the intensity of spots observed. The majority of the poor metabolizers were females while the majority of the extensive metabolizers were males.

Conclusion: Gender differences plays a vital the role in metabolism, therefore outline implementation of phenotype determination before therapy will therefore greatly improve the goal of therapy and quality of life. Implementation of phenotype determination before therapy will, therefore, greatly improve the goal of therapy and quality of life.

Keywords: Chloroquine, Hydroxychloroquine, Adverse effects, Phenotype

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Introduction

Chloroquine, 4-N-(7-chlorquinolin-4-yl)-1-N,N-diethylpentane-1,4-diamine, is usually used for the treatment of malaria, but it has shown some promising effects against COVID-19. Therefore, it is very important to understand how different individual body systems handle this drug, in terms of its metabolism and life-threatening adverse effects. For example, some individuals die as a result of chloroquine accumulation (poisoning) in the system and not necessarily from COVID-19 infection [1]. Human genomic constituents determine how drugs are metabolized and utilized in the body as intended [2, 3]. Chloroquine has many side effects from mild ones like muscle cramps, loss of appetite, diarrhea, and skin abscess, to more serious ones like vision impairment, muscle damage, epileptic-like seizures, and low blood cell levels [4].

A cross-sectional study on healthy adults indicates that the bioavailability of chloroquine is greater when the drug is taken with food rather than in a fasting state. The presence of food does not affect the rate of chloroquine absorption, but peak plasma concentration is higher when the drug is taken with food rather than in a fasting state. The bioavailability of chloroquine is greater when the drug is taken with food rather than in a fasting state. The presence of food does not affect the rate of chloroquine absorption, but peak plasma concentration is higher when the drug is taken with food rather than in a fasting state [5, 6, 7]. Chloroquine and its analogs are rapidly dealkylated by the cytochrome P450 enzymes into pharmacologically active metabolites (desethylchloroquine and bisdesethylchloroquine) [8]. Cytochrome P450 2D6 (CYP2D6) is one of the live enzymes encoded by the CYP2D6 gene in humans [9, 10]. Variations exist in the efficiency and amount of CYP2D6 enzyme produced among different ethnicities or genders. Thus, some individuals will eliminate drugs metabolized by CYP2D6 quickly (ultra-rapid or extensive metabolizers [Ums] or [Ems]), some moderately (intermediate metabolizers [IMs]), while others slowly (poor metabolizers [PMs]). If a drug is metabolized rapidly, its efficacy may be decreased while too slow metabolism will result in toxicity. Therefore, the dose of the drug would be adjusted to account for the speed at which it is metabolized by the enzyme [11]. CYP2D6 shows the largest phenotypical variability among the cytochrome P450 enzymes, which could be due to genetic polymorphism [12].

Thin-Layer Chromatography (TLC) is a simple, accurate, inexpensive, and precise analytical technique used to separate non-volatile mixtures [13]. A compound whose structure resembles the stationary phase will have a low Retardation Factor (Rf), while one with a similar structure to the mobile phase will have high Rf. TLC can be used to monitor the progress of a chemical reaction, identify medicinal compounds present in a given mixture, estimate the concentration of drugs and metabolites in biological fluids (blood, saliva, urine, etc.), and determine the purity of a substance [14-17].

The study aimed to quantify how different individuals metabolize chloroquine as a pointer to a reported adverse reaction, thereby informing clinicians towards the rational use of this drug based on phenotypic variation.

Materials and Methods

Experimental design and sample collection

A survey questionnaire was designed and distributed among potential respondents. The questionnaire contained sections to distinctively assess the compliance of participants to study protocols: section A collects demographic data, section B examines medication/medical history, and section C asks about post-drug surveillance. Samples were collected pre- and post-drug administration. Two collection bottles were given to selected 30 healthy subjects. Two brands of 500 mg chloroquine tablets were administered to 2 groups of healthy volunteers (each group of 15 subjects). Urine samples were collected before the administration of drugs and 2 h after it from volunteers. The samples were extracted with diethyl ether, concentrated, spotted, and eluted on TLC using diethylamine, toluene, and isopropanol combination in the ratio of 1:4:5v/v/v, as mobile phase. The inclusion criteria of the study subjects include being 18 years old and above, not using concurrent medication(s) and willing to comply with study protocols. The exclusion criteria were immunocompromised people taking other medication(s), younger than 18 years, unwilling to follow study protocols, and alcoholics or chronic smokers.

Preparation of standard solution, urine samples, and extraction

An equivalent of 500 mg of powdered chloroquine tablets of Quimal 250 mg (Dana Pharmaceutical Private Ltd Mumbai) and Samquine 250 mg (Sam Pharmaceutical Ltd Nigeria) was separately dissolved in a 20 mL test tube with 4 mL distilled water. To each tube, 6 mL of diethyl ether (Sigma chemicals, USA) was added, stoppered, and shaken gently, and finally allowed to stand to obtain a two-phase system. The organic phase contains the drug that was separated from the aqueous phase to obtain the stock solution. The method employed was based on the report by Joseph et al. [18]. To 1 mL of urine samples (at 25°C), we added 0.3 mL of concentrated hydrochloric acid 37% (JHD of China) with a micropipette. The mixture was then vortexed for 10 s, heated in a water bath at 100°C
for 1.5 h, cooled and finally, 1.4 mL ammonium hydroxide (NH₄OH) (Lobachemie, India) was added to basify the solution. Then, 4 mL diethyl ether (Sigma chemicals, USA) was added and centrifuged at 10000 xg for 30 min. The organic phase was separated from the aqueous phase for TLC analysis. TLC plates were prepared and labeled as A (standard solution), B (drug-free urine), and C (urine after drug administration) and were spotted accordingly, using a different heparinized capillary tube, allowed to dry. Next, the plates were placed in the TLC chamber until the solvent moved to the solvent front, removed, and allowed to dry. TLC plates are viewed under a 254-nm UV lamp (Figure 1). The spots were also identified by developing in an iodine chamber, the invisible spots were made visible by spraying with 10% sulfuric acid, and the intensities of the spots were visually observed and recorded appropriately.

The study was approved by the Research and Ethics Committee of the Faculty of Pharmacy, Niger Delta University, Bayelsa state (Ethical Code: FPH/UG/4642/19).

Results

The obtained data were analyzed using GraphPad InStat 3.0 software, Excel spreadsheet, and Microsoft word version 2013. Data from the different brands of chloroquine phosphate used in the study are presented in Tables 1 and 2 as frequency, percentage, Mean±SD, and in figures as charts.

Discussion

The obtained data were analyzed using GraphPad InStat 3.0, and Microsoft Excel version 2013 windows. The intensities of the spots were carefully observed. About 50% [15] of the study population were male out of whom, 20% [3] were PMs, 60% [9] EMs, and the remaining 20% [3] IMs. In the other 50% [15] females, 40% [6] were EMs, 40% [6] PMs and 20% [3] IMs. This follows Pettersen et al. study results [19] on the variation in metabolism associated with gender disposition that the more muscle, the higher the metabolic rate.

Table 1. Thin layer chromatography retardation factors for chloroquine standard and urine samples collected from 30 volunteers after 2 hours

| Mean Retardation Factor (Rf) of Chloroquine Phosphate in the Urine of Volunteers and Chloroquine Standard | A (S1-S15) Q16-30 | B (Q1-Q15) | C (S1-S15) Q16-30 | A (S1-S15) Q16-30 | B (Q1-Q15) | C (S1-S15) Q16-30 | P |
|-------------------------------------------------------|------------------|-----------|------------------|------------------|-----------|------------------|----|
| S1-15 Q16-30 | S1-15 Q16-30 | S1-15 Q16-30 | S1-15 Q16-30 | S1-15 Q16-30 | S1-15 Q16-30 | S1-15 Q16-30 | A | C |
| 8.5 8.5 4.0 - - - 9.0 10 | 0.94 0.85 - - | 0.9±0.1 0.0±0.0 <0.01* | | | | | |
| 8.5 8.5 _ - - 8.0 9.0 10.0 10 | 0.85 0.85 0.80 0.90 | 0.9±0.0 0.9±0.0 <0.001** | | | | | |
| 8.8 8.5 _ - - 8.5 8.5 10.0 10 | 0.88 0.85 0.85 0.85 | 0.9±0.0 0.9±0.0 <0.001** | | | | | |
| 8.5 8.5 7.5 - - 9.5 8.8 11.0 10 | 0.77 0.85 0.86 0.88 | 0.8±0.1 0.4±0.0 <0.01* | | | | | |
| 8.5 8.5 8.5 - - 8.5 - 10 10 | 0.85 0.85 0.85 - | 0.9±0.0 0.4±0.0 <0.001** | | | | | |
| 8.5 8.5 _ - - 8.5 10 10 | 0.85 0.85 - 0.85 | 0.9±0.0 0.2±0.0 <0.001** | | | | | |
| 8.5 8.5 _ - - 6.0 - 10 10 | 0.85 0.85 - - | 0.9±0.0 0.7±0.2 >0.05 ns | | | | | |
| 8.5 8.5 6.0 - 6.0 8.8 10 10 | 0.85 0.85 0.60 0.88 | 0.9±0.0 0.9±0.1 <0.001** | | | | | |
| 8.5 8.5 _ - - 7.5 9.5 10 10 | 0.85 0.85 0.75 0.95 | 0.9±0.0 0.8±0.1 <0.001** | | | | | |
| 8.5 8.5 _ - - 6.5 7.5 10.0 10 | 0.85 0.85 0.75 0.83 | 0.9±0.0 0.9±0.1 <0.001** | | | | | |
| 8.5 8.5 8.0 - 8.0 10 10 10 | 0.85 0.85 0.80 1.00 | 0.9±0.0 0.8±0.0 <0.001** | | | | | |
| 8.5 8.5 _ - - 8.5 8.5 10 10 | 0.85 0.85 0.85 0.88 | 0.9±0.0 0.9±0.1 <0.001** | | | | | |
| 8.5 8.5 _ - - 8.0 9.0 10 10 | 0.85 0.85 0.80 0.90 | 0.9±0.0 0.4±0.0 <0.001** | | | | | |
| 8.5 8.5 _ - - 7.5 - 10 10 | 0.85 0.85 0.75 - | 0.9±0.0 0.0±0.0 <0.001** | | | | | |

S: Samquine; Q: Quimal; A: Standard drug; B: Drug-free urine; C: urine after drug administration; Rf: Retardation factor; ns: not significant; *Significant; **Highly significant; One-way ANOVA was performed at a P<0.05; The P-value from one-way ANOVA is <0.0001, which is considered extremely significant. Variation among columns means is significantly greater than expected by chance.
fact clearly explains why men have a higher metabolic rate than women. There was no relationship in the rate of drug metabolism with age, although there was no significant difference in their ages. Around 40% [6] of the total female study population were PMs; all of them had experienced a severe adverse reaction (like skin rash, pruritus, etc.). Also, 40% [6] of the female study population were EMs out of whom, 66.67% [4] did not react to the drug. However, 20% [3] of the total male participants were PMs, out of whom, 66.67% [2] had mild adverse reactions like body itch, 33.33% [1] moderate adverse reactions (prolong itching pubic regions), and none severe adverse reaction which could have been due to a higher muscle mass in men compared to women. Sixty percent [9] of the men in the study population were extensive metabolizers, out of whom 44.44% [4] had no adverse reaction while the other 55.56% [5] had either mild, moderate, or severe reaction. This finding was also reported by Aghahowa et al., that pruritus induced by chloroquine was one of the unwanted effects in the pro-

### Table 2. Correlation of demographic data and study outcomes

| Study Code | Sampling Time (h) | Age (y) | Gender | Ethnicity | State of Origin | Adverse Reactions Reported | Thin Layer Chromatography Chromatogram Intensity | Phenotype Classification |
|------------|------------------|--------|--------|-----------|----------------|--------------------------|-----------------------------------------------|-------------------------|
| S1         | 2:31             | 17     | M      | Igbo      | Anambra        | Nil                      | NS                                           | EM                      |
| S2         | 3:00             | 23     | F      | Ijaw      | Bayelsa        | Severe                   | V                                            | PM                      |
| S3         | 8:00             | 19     | F      | Ogbia     | Bayelsa        | Severe                   | L                                            | EM                      |
| S4         | 12:00            | 21     | M      | Hausa     | Bornu          | Mild                     | L                                            | EM                      |
| S5         | 3:30             | 18     | M      | Nenbe     | Bayelsa        | Severe                   | L                                            | EM                      |
| S6         | 2:30             | 20     | M      | Epie      | Bayelsa        | Moderate                 | NS                                           | EM                      |
| S7         | 4:12             | 18     | F      | Igbo      | Anambra        | Nil                      | NS                                           | EM                      |
| S8         | 3:15             | 22     | F      | Ikwere    | Rivers         | Severe                   | V                                            | PM                      |
| S9         | 2:10             | 24     | M      | Yoruba    | Ogun           | Moderate                 | V                                            | PM                      |
| S10        | 6:15             | 21     | F      | Ikwere    | Rivers         | Moderate                 | I                                            | IM                      |
| S11        | 4:10             | 24     | F      | Yoruba    | Ogun           | Severe                   | V                                            | PM                      |
| S12        | 3:15             | 22     | F      | Eleme     | Rivers         | Severe                   | I                                            | IM                      |
| S13        | 2:38             | 26     | M      | Igbo      | Imo            | Mild                     | L                                            | EM                      |
| S14        | 2:52             | 23     | F      | Urhobo    | Delta          | Severe                   | V                                            | PM                      |
| S15        | 3:02             | 27     | M      | Yoruba    | Ogun           | Mild                     | V                                            | PM                      |
| Q16        | 6:00             | 24     | M      | Urhobo    | Delta          | Nil                      | NS                                           | EM                      |
| Q17        | 4:30             | 24     | M      | Nenbe     | Bayelsa        | Nil                      | L                                            | EM                      |
| Q18        | 4:32             | 18     | F      | Okirika   | Rivers         | Severe                   | V                                            | PM                      |
| Q19        | 3:52             | 17     | M      | Igbo      | Abia           | Nil                      | I                                            | IM                      |
| Q20        | 2:06             | 19     | M      | Isoko     | Delta          | Nil                      | NS                                           | EM                      |
| Q21        | 3:00             | 22     | M      | Ogoni     | Rivers         | Severe                   | I                                            | IM                      |
| Q22        | 3:52             | 21     | F      | Isoko     | Delta          | Nil                      | NS                                           | EM                      |
| Q23        | 2:30             | 23     | F      | Ogoni     | River          | Nil                      | L                                            | EM                      |
| Q24        | 4:20             | 25     | F      | Igbo      | Enugu          | Severe                   | I                                            | IM                      |
| Q25        | 3:15             | 24     | F      | Ijaw      | Bayelsa        | Severe                   | V                                            | PM                      |
| Q26        | 2:52             | 20     | M      | Epie      | Bayelsa        | Severe                   | I                                            | IM                      |
| Q27        | 6:15             | 21     | F      | Ogbia     | Bayelsa        | Mild                     | L                                            | EM                      |
| Q28        | 4:10             | 23     | M      | Igbo      | Abia           | Severe                   | L                                            | EM                      |
| Q29        | 3:01             | 25     | M      | Ijaw      | Bayelsa        | Mild                     | V                                            | PM                      |
| Q30        | 2:30             | 20     | F      | Ijaw      | Bayelsa        | Nil                      | NS                                           | EM                      |

Nil: No adverse drug reaction reported; Mild: Experienced just an adverse reaction from chloroquine; Moderate: Subject has experienced two adverse reactions from chloroquine; Severe: Subject has experienced three to four adverse drug reaction from chloroquine;

EM: Extensive Metabolizer; PM: Poor Metabolizer; IM: Intermediate Metabolizer; V: Very intense spot; L: Less intense spot; I: Intense spot; NS: No spots observed.
phylaxis and treatment of uncomplicated malaria. Pruritus has been reported to feel as tolerable to intolerable among susceptible individuals that result in the disruption of treatment and development of resistance to the drug thus leading to therapeutic failures or complications [20]. Thus, adverse reactions tend to be less frequent when lower doses of chloroquine are used. This is the reason for higher chances of developing adverse effects in poor metabolizers than the extensive metabolizers [21]. This gave a glue of what is obtainable globally as a result of the COVID-19 pandemic, so many households have switched into self-medication with chloroquine due

**Figure 1.** Thin layer chromatography plates showing
A: Standard solution; B: Drug-free urine; and C: urine after drug administration

**Figure 2.** Gender-based comparison of the phenotype differences among participants

**Figure 3.** Adverse drug reactions experienced by participants based on gender and nature of reaction(s)
to fear of being infected with the virus. The drug is rapidly accumulated in slow metabolizers, who then have the risk of developing seizures and dying in some cases. The intermediate and extensive metabolizers may not have issues but need to know their status phenotypically before therapy initiation. Also, Figures 2 and 3 show the respective relationship between phenotypic variation and adverse reactions to both brands of chloroquine. There were significant differences in the rate of metabolism and the adverse reaction observed among participants at P<0.05.

Conclusion

The metabolic rate among individuals differs as observed in the study which could be due to variation in gene expression, gender, and concentration of metabolizing enzyme. These variations affect metabolism and ultimately determine the degree of adverse effects related to chloroquine. Phenotypic variation in individuals as a result of gene expression has a significant impact on chloroquine metabolism and ultimately adverse effects as poor metabolizers cannot fully utilize the normal dose. Therefore, chloroquine should only be used as preventative medicine towards COVID-19 and any other condition only if the benefits outweighs the potential risk or after a thorough routine phenotype determination before therapy is initiated, this will improve the goal of therapy and avert chances of potential toxicity.

Ethical Considerations

Compliance with ethical guidelines

The study was performed following strict compliance with Ethical Guidelines and principles. It was approved by the research and ethics committee of the faculty of Pharmacy, Niger Delta University, Bayelsa state with an ethical code: FPH/UG/4642/19. Before recruitment of volunteers, personal consent was also obtained, the study protocols were well explained to each participants.

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Authors' contributions

All authors were equally contributed in preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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