Assessment of Helicobacter pylori positive infected patients according to Clarithromycin resistant 23S rRNA, rpl22 associated mutations and *cyp2c19**1, *2, *3 genes pattern in the Early stage of Gastritis

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Research Article

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Abstract

Objective. Clarithromycin resistant *Helicobacter pylori* (CAM-R) is the main cause of standard triple therapy eradicating failure. Proton pump inhibitors (PPIs) directly poses bacteriocidic activity and prepare the optimum condition for clarithromycin best function. In counter with Poor metabolizer subjects, Homozygote Extensive Metabolizer patients have well characterized within treatment failure. Eventually, determination of CAM-R profile and estimation of PPIs metabolization rate support clinicians in better prescription. This experiment explored mutations in 23S rRNA and *rpl22* resistant genes, *cyp2c19* allele variations *1, *2, *3 and PPIs metabolization patterns, consequently the results reported to the physician.

RESULTS. Sixteen out of 96 patients considered to be CAM-R *Helicobacter pylori*. A2143C (1/16), *rpl22* insertion (16/16), and GTG deletion (2/16) recorded in CAM-R strains. P450 2C19 human genotyping demonstrated that the highest proportion of the *H. pylori* positive strains infected patients 43/61(70.49%) categorized in Homozygote extensive metabolizer class. The rest (12/61)19.66% classified as Poor metabolizer, and 6/61(9.83 %) distinct for Heterozygote extensive metabolizer group. Proportion of poor metabolizer and Heterozygote extensive metabolizer phenotypes between CAM-R strains mentioned to be 10/16(62.5%), and 6/16(37.5%). Cross points between the most frequent distributed allele in CAM-R strains indicated 81.25% for *2, and ^{w}2 for 18.75%.

1. Introduction

Lower efficient *Helicobacter pylori* eradicating by standard triple therapy (STT) has been directly related to incresement of clarithromycin resistance rate (CAM-R) [1]. Nevertheless, many compensatory mutations, 23S rRNA related point mutations A2142G/C, A2143G/C and recently *rpl22* polymorphisms (GTG deletion and TTCCATGTA insertion) individually discussed among CAM-R strains [2-4].

Proton pumps inhibitors (PPIs) covalently interact with cysteine residue of proton pumps which inhibit H^{+} releases, thereby collaboration of PPIs in prescription promote stability and concentration of Clarithromycin. P450 CYP 2c19 is the liver catabolic enzyme that dominantly corresponded in metabolization of omeprazole and lansoprazole [5-7]. Among 34 *cyp2c19* allele polymorphisms distinct for the deficiency in drug metabolization, there are three major loss of functions (LOF) *cyp2c19**2 (681 G≥A), *cyp2c19**3(636 G≥A), and *cyp2c*19*17 (806 C≥T) in which *cyp2c19**2 and *cyp2c19**3 are mainly reputed in Asian population than *cyp2c19**17 for less than 1% [7].

Based on the *cyp2c19* variants, subjects have been categorized in to three groups: Homozygote extensive metabolizer (Hom-EM) with two wild types allelic polymorphism, Heterozygote extensive metabolizer (Het-EM) with LOF *2 or *3, and poor metabolizer (PM) with two losses of function *2 and *3 [8]. Furuta was the pioneer in evaluation of *cyp2c19* human genotyping and prediction of cure rate after treatment regime consumption. Beyond the series of randomized Clinical trial studies eradication rate in PM group that taken standard dosage pf PPIs considered to be high and in EM participants reported to be very low, so presumption of the infection recurrence comes to be high [9, 10].

Thus, to support clinicians in better scheduling we performed PCR sequencing to evaluate CAM-R related point mutations in 23S rRNA and *rpl22* genes, and Realtime-PCR in classification of total patients in the early stage of gastritis, *Helicobacter pylori* positive infected individuals, Clarithromycin resistant strains infected patients based on *cyp2c19* gene mapping.

2. Main Text

2.1 Material and methods

Total of 96 consenting participants were concluded in this associational study, during the period of April 5th, 2020 to October 9th, 2020. *H. pylori* phenotypically and molecular characterization, bacterial phenotypically antimicrobial drug resistance (ADR), subsequently 23S rRNA and *rpl22* polymorphisms confer CAM-R determined by PCR sequencing are subscribed in additional file 1 and 2. To detect *cyp2c19* *1, *2 and *3 LOF Primer designing were clarified in additional file3. Reagents preparation and RT-PCR performance in differentiation of total patients, *H. pylori* positive subjects, CAM-R strains infected one according to *cyp2c19* mentioned varriants described in additional file4. Additional file5 have contented *cyp2c19* *1, *2, *3, 23S rRNA, and *rpl22* pair primers list.

3. Statistics Analysis

The SPSS Statistics for Windows (version 21.0, IBM Corp, Armonk, NY, USA) and Chi-Square (χ2) Definition applied to search for associations between the variables and classification of the population studied; *p*-values ≤0.05 was considered to be significant range in interpretation.

4. Results

Describes the characteristic of the patients from whom *H. pylori* strains were isolated by histopathology test, molecular identification, and bacterial culturing illustrated in additional file6.

4.1 *Cyp2c19**1, *2, and *3 allele distribution

According to our experimental study, frequent allelic polymorphism through *cyp2c19* *1(which is the wild type) *2 and *3, denoted for ^{w}3 (81%) *p*≤0.001, was circulated intra total number of enrolled patients. The next were*2 for (13.5%) *p*-value ≤0.001, ^{w}2 (3.1%) *p* ≤0.75 and *3(2.08%) *p* ≤0.75.

According to the present study, the frequency of *3 and ^{w}2 in distribution were the lowest but more than 1%. Chi-Square (χ2) Statistic analysis reports of *cyp2c19* *1 variants distributed in the total patients attended in this work (as the reference group) in comparison to histopathological positive *Helicobacter pylori*, molecular positive *H pylori*, and culture positive group considered to be significant *p*-value≤ 0.001, and in CAM-R strains reported for *p*= 0.75. *cyp2c19* *2 allele-span through mentioned classified groups considered to be significant *p*-value- ≤ 0.001 and circulation of *cyp2c19* *3 within total examined subjects noted for *p*≤ 0.75.

4.2 Accumulation of *cyp2c19* *2 variants among phenotypically and molecular characterized CAM-R strains

Spanning of the *cyp2c19* *1, *2, and *3 allelic polymorphism inter culture positive *Helicobacter pylori* strains demonstrated the prevalence of ^{w}3 (70.49%) *2(21.30%) and ^{w}2 (4.9%) and *3(3.27%), totally. The dominant allelic-polymorphism through CAM-resistant strains 81.25% was recorded for *2 *p*-value ≤0.001, and 18.75% for ^{w}2 *p* ≤0.75, respectively. Because of accumulation of *rpl22* 9bp insertion, *rpl22* 3bp deletion and the only one A2143C point mutations related to CAM-resistance in *2 PM, and *2 Het-EM metabolizer class; therefore, it is clear that the cross point between the most frequent allele that distributed in CAM-R strains will be *2(81.25%), and ^{w}2 for 18.75%. Distribution of ^{w}3 and *3 among CAM-resistant strains noted to be zero, Fig 1.

4.3 Characterization of PPIs catabolization pattern within the patients totally

The release of our experiment demonstrated that through the total number of the patients (n=96), there were 81.2% distinct for Homozygor extensive PPIs metabolizer with the allelic pattern of (^{w}3/^{w}3), 6.25% of the patients classified in Het-EM with the allelic pattern of (^{w}2/*2) and the rest of 12.48% have been characterized for poor metabolizer category,

with the allelic pattern of (*2/*2) for 10.4% and (*3/*3) for 2.08%. The pattern of (^{w}3/*3) Het-EM was not obviously detected in our experiment.

4.4 *Cyp2c19* phenotype distribution between infected individuals

Comparative analysis of the total number of the patients demonstrated that 63/96 histopathological examined patients and 61/96 molecular identified patients were *H. pylori* positive. Distribution of the Hom-EM (^{w}3/^{w}3), Het-EM (^{w}2/*2) and PM phenotypes in infected individuals by histopathological and molecular tests reports, were ordinarily 65.6%, 9.52%, 19.4% and 70.49%,9.83%, and 19.66%. Poor metabolization pattern within the Histopathological reported *H. pylori* positive patients were (*2/*2)15.87%, (*3/*3) 3.17% and for molecular identified patients recorded 16.39% for (*2/*2) and 3.27% for (*3/*3).

4.5 PPIs metabolizer phenotype patterns and profile of CAM-resistant

In the manner of *cyp2c19* gene dosage profiling between CAM-R strains that circulated through the population with the perspective of personalized therapy; replacement of the drug, duration or drug dosing; first, 35/96 (37%) of the individual phenotypically evaluated *Helicobacter pylori* positive, that the prevalence of Hom-EM participants (^{w}3/^{w}3), Het-EM(^{w}2/*2) and PM (*2/*2) vs (*3/*3) were reported 48.57%, 17.14%, 28.57 and 5.7 % ordinarily. Already Details of results accumulated in Table 1. In this survey, there was significant coverage between CAM-resistant strains 16/35(37%) and distribution of two phenotypes of PPIs metabolization rate: 62.5% for PM (*2/*2) and 37.5% for Het-EM (^{w}2/*2). The more interesting notification of our results was the accumulation of the total number of the point mutations (A2143C and rpl22 GTG deletion or 9bp insertion) correlated with the CAM-R strains in two phenotypes: PM (*2/*2), and Het-EM(^{w}2/*2). According to our study from (10/16) 62% of poor metabolizer patients were characterized for allelic pattern of (*2/*2); spanning of CAM-R related point mutations noted to be: 1/10 for A2143C, 8/10 for *rpl22*9bp insertion, and 2/10 for *rpl22* GTG deletion and 9bp insertion. The molecular pattern of the rest of 6/16 (37%) CAM-R isolates with *rpl22* 9bp insertion, classified in Het-EM Table 2.

5. Discussion

According to Kyoto global consents report initial molecular approaches in patients screening according to CAM-R profile and PPIs-metabolization rate improve the rate of eradication [11]. Indeed, this experiment performed to evaluate the local profile of Clarithromycine resistant strains infected patients consequently *cyp2c19*1,* *2, *3 patients pattern in PPIs (omeprazole and lansoprazole) metabolization rate.

Based on our survey, the most proportion of the patients, total number of the examined patients, histopathology and molecular infected evaluated individuals and culture positive patients, classified in Hom-EM class (cyp2c19*1) *P*-value≤ 0.001, that supported by Mahmoudi Saber et al. [12] in Tehran, that the rate of Hom-extensive metabolizer patients reported for 85.9%. Didevar et al. [13] by investigating the Azari Turkish healthy individuals, demonstrated the most content of the subjects categorized in Hom-EM group. According to our work, the rest of the patients were classified in PM (12.8%) and Het-EM (6.25%). *cyp2c19* *2*3 allelic pattern distributed in PM and Het-EM groups noticed the higher rate of *2 allele *p*≤ 0.001 distribution than*3 *p*-value≤ 0.75, in mapping. A comprehensive review of Iranian *cyp2c19* Gene Polymorphisms Population reported the spanning of *2 variants (13.6%) that the prevalence was obviously in a row with our work [14], they have been reported *3 allelic variations with the minor allele frequent class (MAF) ≤1% that based on our experiment the prevalence of *3 allelic polymorphism described spanning in limited subjects 2.08% *p*≤ 0.75. Based on our findings, Het-EM patients allelic combination exhibited ^{w}2/*2 pattern in diagnosis, which was in order with Saber et al. [15] In both studies the association of Het-EM patients with the ^{w}3/*3 pattern was considered to be zero. Illustration of the dominant *2/*2 structure of PM patients in our study revealed the similarity to, Saber et al. [12], Namazi et al. [15],

Zendehdel et al. [16], and whom reported the relation between the dominant *2/*2 pattern in PM patients. However, in our experiment small portion of PM phenotype was considered for *3/*3 pattern that in their study, such combination was noticed to be zero.

Predicted on a series of studies exceeded the rate of Clarithromycin resistance up to 15%, and replacement of Standard triple therapy by Bismuth quadruple therapy, hybrid (or reverse hybrid) therapy, and concomitant therapy are on the double scale in the prescription [17]. Case-to-case therapy by determination of CAM-R pattern and *cyp2c19* polymorphisms improver the superior in quality, with fewer adverse events [18]. In Turkey, consequences of CAM-R strains rose rate to 40% linked to decreases of STT efficiency for 55.7% [9]. Choi et al. [18] indicated that 23S rRNA point mutations monitoring in CAM-R strains increases the eradication rate from 82.6% to 91.2%. Nor efficiency improvement but also the rate of eradication related side effects decreased for 12.0%, that significantly looks differ from empirical bismuth quadruple therapy for *H. pylori* first-line eradication regime.

To pretreatment ideally therapy, these findings led us to categorize the patients based on clarithromycin resistant and pattern of PPIs metabolization rate, and results reported to the physicians. The findings demonstrated that 100% of the phenotypically CAM-R strains covered by *rpl22* mutations. According to us all the Clarithromycin sensitive patients classified in Homozygote extensive metabolizer group, and all the CAM-resistant patients described for PM 62.5% and Het-EM 37.5%, respectively. It is worth to mention that accumulation of all CAM-R strains, CAM-R related point mutations in *cyp2c19**2 variant were significant *p*-value≤ 0.001; *rpl22* 9bp insertion, *rpl22* 3bp deletion and the only one A2143C point mutations related to CAM-resistance all categorized in *2 PM, and *2 Het-EM metabolizer class. Yi Song et al. In a similar experience denoted that the distribution of the individual's in Het-EM, EM, and PM groups were 53%, 38%, and 9%; The most interesting notification of their datas were the accumulation of the CAM-R related point mutations in EM class [19], that were in counter with our findings.

Based on the results definition the majority of the individuals and all the CAM-S strains classified in the EM group, by that means before the beginning of the therapy it is so clear that the recurrence of the infection in the subjects that received standard PPIs (dosing or duration) seems to be high. All of CAM-R patients were categorized in PM62.5%, and Het-EM 37.5% groups that the prediction of cure rate by standard PPI_S dosage scheduling in PM groups seems to be fine and controversial in Het-EM group.

Limitations

In the purpose of initial tailoring therapy, we introduced three groups of patients to clinicians: first, CAM-R infected subjects (Tagged for 23S rRNA and *rpl22* related point mutations) for drug replacement or alternative treatment regime in use; second, PM patients in CAM-S group (that may suffer from long-term PPIs and side effects), and CAM-R groups; finally, EM patients that the risk of infection recurrence by standard PPIs dose scheduling consider to be very high. Short time to patients follows up project, a need for an online system for requesting follow-up appointments, and physician's persistence in empirical therapy than per patient therapy could be our limitation in this experiment.

Abbreviations

CAM-R: clarithromycin resistant

PPIs: Proton pumps inhibitors

STT: standard triple therapy

LOF: loss of functions

Hom-EM: Homozygote extensive metabolizer

Het-EM: Heterozygote extensive metabolizer

PM: poor metabolizer

ADR: antimicrobial drug resistance

WHO: world health organization

RCTs: randomized controlled trials

MAF: minor allele frequent

Declarations

Contributions:

AAM and AMM developed the idea, designed the study, AAM, AS and AY collected the samples, AAM, AMM, SE and MN analyzed the data and drafted the manuscript. AMM reviewed and revised the manuscript. All authors read and approved the final manuscript.

Competing interest:

The authors declare that they have no competing interests.

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Ethics Approval and Consent for publication:

This survey was approved by the Ethics Committee of Tarbiat Modares University (IR-MODARES.REC.1398/019), Tehran, Iran; all the participants have accepted and signed the informed consent.

Availability of data and materials:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Not applicable

References

1. Graham DY, Shiotani A. New concepts of resistance in the treatment of *Helicobacter pylori* infections. Nat Clin Pract Gastroenterol Hepatol. 2008;5(6):321-31. doi: 10.1038/ncpgasthep1138
2. Tuan VP, Narith D, Tshibangu-Kabamba E, Dung HDQ, Viet PT, Sokomoth S, et al. A next-generation sequencing-based approach to identify genetic determinants of antibiotic resistance in Cambodian *Helicobacter pylori* clinical isolates.

J Clin Med. 2019;8(6):858. doi: 10.3390/jcm8060858

3. Binh TT, Shiota S, Suzuki R, Matsuda M, Trang TTH, Kwon DH, et al. Discovery of novel mutations for clarithromycin resistance in *Helicobacter pylori* by using next-generation sequencing. J Antimicrob Chemother. 2014;69(7):1796-803. doi: 10.1093/jac/dku050
4. Miftahussurur M, Syam AF, Nusi IA, Makmun D, Waskito LA, Zein LH, et al. Surveillance of *Helicobacter pylori* antibiotic susceptibility in Indonesia: different resistance types among regions and with novel genetic mutations. PloS one. 2016;11(12):e0166199. doi: 10.1371/journal.pone.0166199
5. Kuo C-H, Lu C-Y, Shih H-Y, Liu C-J, Wu M-C, Hu H-M, et al. *CYP2C19* polymorphism influences *Helicobacter pylori* eradication. World Journal of Gastroenterology: WJG. 2014;20(43):16029-16036. doi: 10.3748/wjg.v20.i43.16029
6. Yang JC, Yang YF, Uang YS, Lin CJ, Wang TH. Pharmacokinetic–pharmacodynamic analysis of the role of *CYP2C19* genotypes in short-term rabeprazole-based triple therapy against *Helicobacter pylori*. Br J Clin Pharmacol. 2009;67(5):503-10. doi: 10.1111/j.1365-2125.2009.03393.x
7. Arévalo-Galvis A, Otero-Regino WA, Ovalle-Celis GN, Rodríguez-Gómez ER, Trespalacios-Rangel AA. Prevalence of *CYP2C19* polymorphism in Bogotá, Colombia: The first report of allele* 17. Plos one. 2021;16(1):e0245401. doi: 10.1371/journal.pone.0245401
8. Peng X, Song Z, He L, Lin S, Gong Y, Sun L, et al. Gastric juice-based real-time PCR for tailored *Helicobacter pylori* treatment: a practical approach. Int J Med Sci. 2017;14(6):595-604. doi: 10.7150/ijms.18996
9. Kaplan M, Tanoglu A, Duzenli T, Tozun AN. *Helicobacter pylori* treatment in Turkey: Current status and rational treatment options. Northern clinics of Istanbul. 2020;7(1):87-94. doi: 10.14744/nci.2019.62558
10. Furuta T, Sugimoto M, Kodaira C, Nishino M, Yamade M, Shirai N, et al. Personalized medicine for eradication of *Helicobacter pylori*. Per Med. 2007;4(3):321-328. doi: 10.2217/17410541.4.3.321
11. Yang L, Zou A, Wu H, Guo H, Zhang F, Zou B, et al. Application of Visual Gene Clip-Based Tailored Therapy for the Eradication of *Helicobacter pylori*. Biomed Res Int. 2021;2021:6150628. doi: 10.1155/2021/6150628
12. Saber MM, Boroumand M, Behmanesh M. Investigation of *CYP2C19* allele and genotype frequencies in Iranian population using experimental and computational approaches. Thromb Res. 2014;133(2):272-5. doi: 10.1016/j.thromres.2013.11.005
13. Didevar NA, Niaei G, Hagh MF, Taghavi BA. Cytochrome P4502C19* 3 allelic variant frequency in Iranian healthy Azeri Turkish population. *J Anal Res Clin Med.*. 2016;4(2):110-4. doi: 10.15171/jarcm.2016.018
14. Neyshaburinezhad N, Ghasim H, Rouini M, Daali Y, Ardakani YH. Frequency of important cyp450 enzyme gene polymorphisms in the iranian population in comparison with other major populations: a comprehensive review of the human data. J Pers Med. 2021;11(8):804. doi: 10.3390/jpm11080804
15. Namazi S, Kojuri J, Khalili A, Azarpira N. The impact of genetic polymorphisms of *P2Y12, CYP3A5 and CYP2C19* on clopidogrel response variability in Iranian patients. Biochem Pharmacol. 2012;83(7):903-8. doi: 10.1016/j.bcp.2012.01.003
16. Zendehdel N, Biramijamal F, Hossein-Nezhad A, Zendehdel N, Sarie H, Doughaiemoghaddam M, et al. Role of cytochrome P450 2C19 genetic polymorphisms in the therapeutic efficacy of omeprazole in Iranian patients with erosive reflux esophagitis. Arch Iran Med. 2010;13(5):406-12. URL: www.aimjournal.ir/Article/90
17. O'Connor A, Liou JM, Gisbert JP, O'Morain C. treatment of *Helicobacter pylori* infection 2019. Helicobacter. 2019;24:e12640. doi: 10.1111/hel.12640
18. Choe AR, Shim K-N, Park Y, Song E-M, Tae CH, Jung S. Cost-effectiveness, efficacy, and safety analysis of tailored therapy in patients with *Helicobacter pylori* infection. J Clin Med. 2021;10(12):2619. doi: 10.3390/jcm10122619
19. Song Y, Dou F, Zhou Z, Yang N, Zhong J, Pan J, et al. Microarray-based detection and clinical evaluation for *Helicobacter pylori* resistance to clarithromycin or levofloxacin and the genotype of CYP2C19 in 1083 patients.

Tables

Table 1. Descriptive analysis of p450 2c19 *1, *2, *3 human genomic pattern and allelic variation

Cyp2c19 polymorphisms	Total number of gastritis patients	Histopathological infected report	*H. pylori* positive molecular report	*H. pylori* Culture positive patients	CAM-R *H. pylori*
$^{w}3/^{w}3$	78/96(81.2%)	45/63(65.6%)	43/61(70.49%)	17/35(38.57%)	–
*2/*2	10/96(10.4%)	10/63(15.87%)	10/61(16.39%)	10/35(28.57%)	10/16(62.5%)
$^{w}2/*2$	6/96(6.25%)	6/63(9.52%)	6/61(9.83%)	6/35(17.14%)	6/16(37.5%)
*3/*3	2/96(2.08%)	2/63(3.17%)	2/61(3.27%)	2/35(5.71%)	–
*1	84.32%	70.36	75.40%	47.14%	18.75%
*3	2.08%	3.17%	3.27%	5.71%	–
*2	13.5%	20.63%	21.30%	37.14%	81.25%
	N=96	N=63	N=61	N=35	N=16

Table 2. Cross reaction between *cyp2c19* allelic variations and profile of mutations in CAM R isolates

Target gene	Mutation	PPIs metabolization Phenotype	Metabolization pattern	Total number of CAM-resistant strains
23SrRNA	A2143C transition	PM	*2/*2	1/16
rpl22	TTCCATGTA insertion	PM	*2/*2	10 /16
rpl22	TTCCATGTA insertion	Het-EM	$^{w}2/*2$	6/16
rpl22	GTG deletion	PM	*2/*2	2/16

Figures

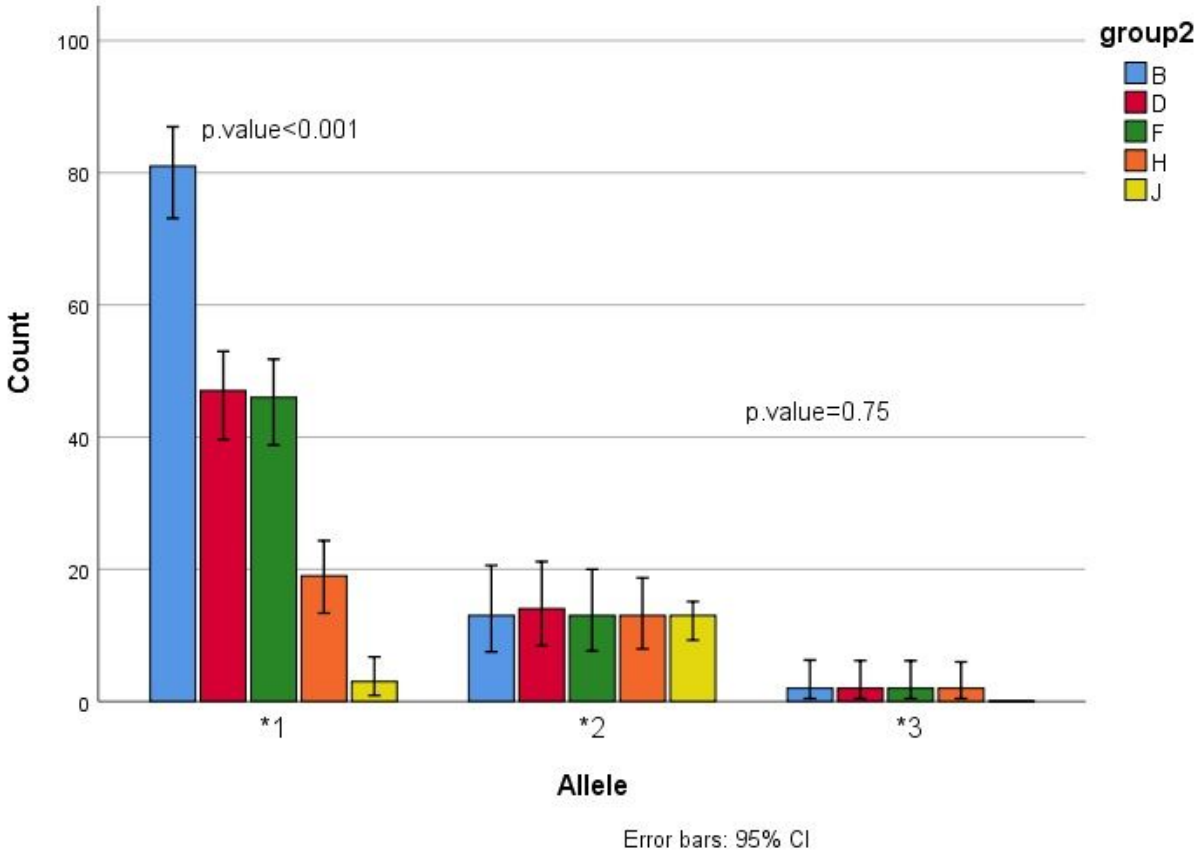

Figure 1

Schematic distribution of cyp2c19 *1, *2, *3 alleles-frequency. Group (B) recognized as the total gastritis patients, (D)Histopathological examined infected group, (F) Molecular characterized H pylori strains infected patients, (H) culture positive H pylori, and (J) CAM-R strains infected individuals. Statistical report of cyp2c19 *1 in BDFH considered in significant range p-value ≤0.001and in CAM-R strains reported for p =0.75. cyp2c19 *2 spanning through BDFHJ considered reliable p ≤0.001. cyp2c19 *3 circulation in BDFH groups were p ≤0.75.

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