Original Article

PHARMACOKINETIC PARAMETERS OF AMOXICILLIN IN BANGLADESHI VOLUNTEERS: A PRELIMINARY EVALUATION

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ABSTRACT

The present study was designed to get preliminary idea about the pharmacokinetic behavior of the Bangladeshi population through estimating plasma amoxicillin concentration by High-Performance Liquid Chromatography (HPLC) with ultraviolet detection. In this study, Bangladeshi healthy volunteers were divided in two groups, 8 Bangladeshi Bangalee and 7 Bangladeshi Tribal male healthy volunteers. Both the groups received 500 mg of amoxicillin in oral route and blood samples were collected at 0, 30, 60, 120, 180, 360 and 480 minutes after drug administration. After 1 week of washout period, same volunteers of two groups received 500 mg of amoxicillin in intravenous route. In case of oral route, the C_{max}, $AUC_{0–8h}$, T_{max} and $T_{1/2}$ values for Bangladeshi Bangalee and Tribal healthy volunteers were 6.78 ± 1.20 & 9.10 ± 1.34 ìg/mL, 1290.13 ± 158.39 & 1766.06 ± 188.37 ìg min/mL, 82.50 ± 32.05 & 102.86 ± 29.28 min and 96.05 ± 3.80 & 88.15 ± 5.33 min respectively. The difference in C_{max}, $AUC_{0–8h}$ and $T_{1/2}$ values between these two groups of volunteers was significant ($p<0.01$, $p<0.001$ and $p<0.01$ respectively). However, the difference in T_{max} was not significant ($p>0.05$). In case of intravenous route, the $C_{30\ min}$ and $AUC_{0–8h}$ values for Bangladeshi Bangalee and Tribal healthy volunteers were 17.88 ± 1.14 & 18.58 ± 0.71 ìg/mL, 2297.96 ± 222.49 & 2376.41 ± 149.99 ìg min/mL respectively and the difference was not significant ($p>0.05$). The $T_{1/2}$ for Bangladeshi Bangalee and Tribal healthy volunteers were 97.50 ± 3.33 & 94.40 ± 2.33 min respectively and the difference was significant ($p<0.05$).

The Mean Percent Absolute Bioavailability in Bangladeshi Bangalee and Tribal healthy volunteers was 56.76 ± 5.39 and 74.17 ± 3.90 respectively and the difference was highly significant ($p<0.001$).

The study concluded that the pharmacokinetic parameters of amoxicillin significantly varied among Bangladeshi Bangalee and Bangladeshi Tribal healthy volunteers indicating necessity of further study on population pharmacokinetic to formulate tailor-made drug therapy in these groups of people.

Keywords: *Amoxicillin; Bangladeshi Bangalee healthy volunteers; Bangladeshi Tribal healthy volunteers; Pharmacokinetic parameters; Bioavailability.*

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INTRODUCTION

Disease pattern of least developed countries is different from those of the developed countries. Until now, infections are the predominant reason of morbidity and mortality in these countries. Therefore, antimicrobials account for a significant proportion of drug consumption of developing as well as least developed countries like Bangladesh[1-5]. On the top of that, emergence of resistance has worsened the situation imposing negative impact on health and ecology. These issues collectively contributed to a situation, where antimicrobials warrant immediate attention as the most important drug to watch in a country like Bangladesh[2, 6-7]. For this purpose, detail understanding is required about local, regional, and national antimicrobial susceptibility surveillance data to support selection of appropriate agents[8]. Though there are few studies on susceptibility pattern, however, nothing is known about pharmacokinetic status of the Bangladeshi population regarding the antimicrobials.

Among the antimicrobials used in different health facilities of Bangladesh, amoxicillin is one of the most commonly consumed one[2-3], possibly because of its favorable spectrum, absorption and tolerability[9]. There are evidences that the antibacterial activity of amoxicillin

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is superior over other â-lactam antibiotics[10-11]. The commonly recommended oral dosage for adult patients is 250–500 mg three times a day[12-13]. Presence of food do not interfere absorption or influence plasma concentration of amoxicillin[14]. The plasma protein binding of amoxicillin ranges from 17% to 20% and its excretion is predominantly renal[15]. Following oral administration, it is rapidly absorbed, metabolized and excreted in the urine and bile[16].

Studies conducted in different countries provided information about the pharmacokinetic behavior of that studied population. There were efforts to correlate plasma concentration with clinical outcome, though very little progress has been made so far[17-18]. Inadequate information about the pharmacokinetic status of Bangladeshi people led to necessity of research to enable scientists to understand these issues.

Though there were different methods to estimate amoxicillin in plasma[19-23], however, none was executed in Bangladesh and therefore the initial challenge was to establish a suitable method in laboratories of Bangladesh. Therefore, the aim of this study was to establish a method of estimation of amoxicillin as well as to get preliminary idea about the pharmacokinetic behavior of Bangladeshi healthy volunteers of two different ethnic origins.

MATERIALS AND METHODS

Subject population

The present study included Bangladeshi healthy volunteers of two categories e.g., Bangalee and Tribal. For the purpose of categorization, the family and anthropometric history was considered. This study included 8 (eight) Bangladeshi Bangalee male healthy volunteers, aged 23–25 years (23.9 ± 0.8 years), weighing between 50–60 kg (55.5 ± 4.0 kg) & within 15% of the ideal bodyweight and 7 (seven) Bangladeshi Tribal male healthy volunteers, aged 23–25 years (24.1 ± 0.7 years), weighing between 50–60 kg (54.1 ± 3.7 kg) & within 15% of the ideal bodyweight. Volunteers were screened to ensure that they have no cardiac, renal, hepatic, hematological, neurological, gastrointestinal and pulmonary disorders and allergy to penicillin.

The volunteers were requested to stay away from any medication for 2 weeks prior to the study and up to its completion. Moreover, they were requested not to take any beverages like alcohol, coffee and tea in 48 h prior to first dose and until the collection of last blood sample[24].

Ethical clearance

The protocol of this study was approved by the National Research Ethics Committee of Bangladesh Medical Research Council (BMRC/NREC/2007-2010/1709). Informed written consent was obtained from all participants after explaining the nature, risk and benefits of the study to them.

Drugs

Commercially available original preparation of Amoxicillin 500 mg injections and capsules were used.

Clinical protocol

This study was conducted in an open label design with one-week washout period between intravenous and oral doses. Initially, all volunteers received oral dose of 500 mg amoxicillin. After one-week wash period, the same volunteers received intravenous dose of 500 mg amoxicillin capsule. The volunteers were observed by experienced physician to detect adverse effects (if any) during the study.

Blood sample collection

For collection of blood samples, a catheter in situ was placed in one arm by skilled nurse in presence a physician with special expertise. The catheter was removed immediately after the study requirement. Seven blood samples (3 ml at each occasion) were obtained at 0, 30, 60, 120, 180, 360 and 480 minutes after drug administration and placed in sterile tubes with 100 ìL of 10% EDTA solution. Immediately after each blood collection, the samples were centrifuged at 3000 × *g* for 15 min and plasma was then separated and stored at "70°C. The stored samples were then studied by HPLC in appropriate method[19-20].

Sample analysis

Amoxicillin plasma concentrations were measured by high-performance liquid chromatography (HPLC). Specificity, linearity, lower limit of quantification, inter-day and intra-day precision and accuracy as well as absolute recovery and stability of amoxicillin was evaluated.

Chromatographic analysis

Amoxicillin analysis was performed using previously adapted and validated methods[19-20].

Chromatographic Condition

HPLC: Alliance HPLC, Origin: Waters USA (Separation Module, Model: Waters 2695; Detector, Model: Waters 2487 and Empower software); Column: Symmetry C18 (2.5 cm x 4.6 mm), Waters-USA; Flow rate: 1.30 mL/min ; Run Time: 7 min ; Column Temperature: Ambient; Detector: UV detector; Test wave length: 229 nm; Cell temperature: 40^0C; Injection Volume: 20µL

Reagents

Sodium Dihydrogen Phosphate Dihydrate (MW=156.01) of Analytical Reagent Grade and Acetonitrile of HPLC Grade was used.

Preparation of Phosphate Buffer, pH = 4.8

1.560 g of $NaH_2PO_4.2H_2O$ (Wt = 156.01 g) was taken in 1000 ml Volumetric Flask. Then was dissolved, diluted and volume increased up to mark with water. The pH was adjusted at 4.8 with dilute NaOH solution or dilute phosphoric acid solution.

Preparation of Mobile Phase

Buffer: Acetonitrile = 95: 5

Preparation of Diluent for Stock solution and Working Standard

Water: Acetonitrile = 95: 5

Preparation of Stock Solution of Amoxicillin (Concentration: 1 mg/mL i.e. 1000 µg/ml)

0.115 g of Amoxicillin Trihydrate (equivalent to 100 mg of Amoxicillin) was taken in 100 mL volumetric flask. Then that was dissolved, diluted and volume increased up to mark with diluent (Water: Acetonitrile = 95: 5).

Preparation of Working Standard from Stock Solution (Concentration: 1000 µg/mL)

Working Standard **1 µg/mL**: 0.1 mL of stock solution was taken in 100 mL volumetric flask, diluted with diluent (Water: Acetonitrile = 95: 5) and then volume increased up to mark.

Working Standard **10 µg/mL**: 1.0 mL of stock solution was taken in 100 mL volumetric flask, diluted with diluent (Water: Acetonitrile = 95: 5) and then volume increased up to mark.

Working Standard **100 µg/mL**: 10.0 mL of stock solution was taken in 100 mL volumetric flask, diluted with diluent (Water: Acetonitrile = 95: 5) and then volume was increased up to mark.

Preparation of Cefadroxil Solution

107 mg of Cefadroxil (equivalent to 100 mg of Cefadroxil) was dissolved in 100 mL volumetric flask, diluted with Methanol and later volume increased up to the mark, getting a concentration of 1 mg/mL i.e., 1000 µg/mL. 10 mL of this solution was taken in another 100 mL volumetric flask and volume increased up to mark with methanol (100 µg/mL). Then 0.15 mL of this solution was taken and 0.85 ml of methanol was added to make 1 mL of Final solution (15 µg/mL).

Preparation of sample Solution:

0.1 mL of plasma was mixed with 0.90 ml of MeOH to make 1.0 mL of sample solution.

Injection Steps

Step 1: 1 mL of drug free plasma + 9 mL MeOH = 10 mL solution as a blank

Step 2: 1 mL of drug free plasma + 1 mL Cefadroxil (15 µg/mL) + 8 ml MeOH = 10 mL solution

Step 3: 1 mL of drug free plasma + 1 mL of Amoxicillin (20 µg/mL) + 1 ml Cefadroxil (15 µg/mL) + 7 mL MeOH = 10 mL solution

Step 4: Working Standard

Step 5: Samples

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the samples containing amoxicillin and internal standard (Cefadroxil) with those obtained from blank samples.

Calibration and validation

Standard Amoxicillin samples were prepared in control plasma using standard stock solutions of Amoxicillin (1.00, 10.00 and 100.00 µg/mL). The overall coefficient of correlation between absorbance and standard drug concentrations was 0.99+0.009 and the standard curve obtained was linear and followed Beer's law from 0.07 to 1.15 µg/mL. The overall recovery of Amoxicillin from plasma standard solutions was 98.85$\pm$1.86 per cent with a range of 98.85 to 100.41 per cent. The inter-day coefficient of variation of the recovery from standards in the above mentioned range was 2.21$\pm$1.83 per cent.

Pharmacokinetic and statistical analysis

Maximum observed plasma concentration (C_{max}) and time taken to reach it (T_{max}) were obtained from drug concentration vs. time curves, which was applicable for oral route only. In case of intravenous route, concentration obtained at 30 minutes was mentioned as $C_{30\ min}$ and therefore T_{max} was not shown. The areas under the amoxicillin concentrations vs. time curves from 0-8 hours (AUC_{0-8h}) were calculated using the linear trapezoidal method and $AUC_{infinity}$ value was not calculated as the remaining constitutes very insignificant part. $T_{1/2}$ was calculated through determining k_{el} considering the linear part of the curve extending from the peak. C_{max} and AUC_{0-8h} data were analyzed statistically using one-way ANOVA and Student's t-test. The researcher has developed a non-compartmental pharmacokinetics data analysis Excel template to calculate and analyze the parameters. Statistical analysis was performed using SPSS version 15.

RESULTS

All volunteers completed the study without any event, which was ascertained by thorough medical examination after study completion.

HPLC was sensitive in quantifying amoxicillin in plasma. The limit of detection (LOD) and limit of quantification (LOQ) was 0.1 and 0.3 µg/mL respectively and the calibration curve was linear over the range of 1.00 to 100.00 µg/mL with a regression coefficient R^2 = 0.9996. Fig 1 shows the calibration curve:

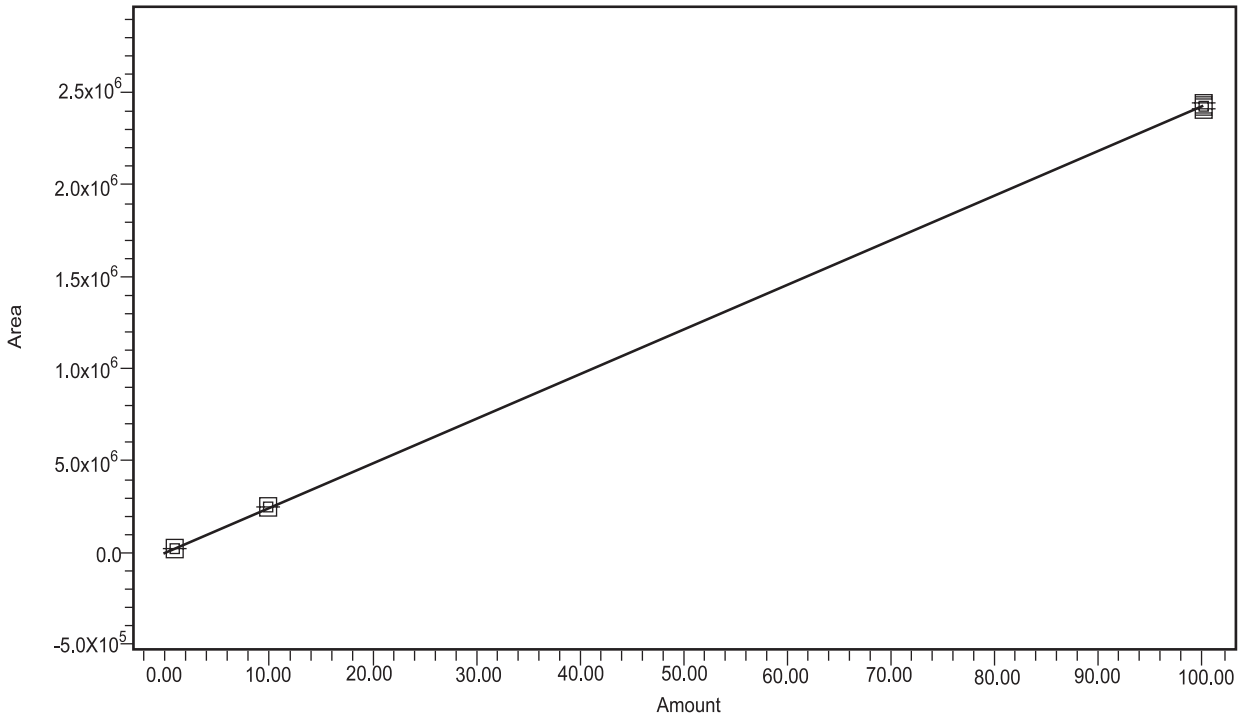
Empower software

Lc Calibration report Sample

Reported by User: reefat

Project name : Amoxicillin_CPR

Processing Method:	Amoxicillin_calibration	Project Name:	Amoxicillin_CPR
Processing Method:	4823	System:	QC_HPLC
Calibration ID:	4816	Channel:	2487Channel 1
Date Calibrated:	3/25/2010 2:07:42 PM	Proc. Chnl.	Descr.:



Name: Amoxicillin; RT: 3.442; Fit Type: Linear (1st Order)(; Cal Curve Id: 4817; R:0.999998; R^2: 0.999995; Weighting: none: Equation: Y = 2.44e+004 X + 3.28e+003

Peak: Amoxicillin

	Sample Name	Result Id	Name	Level (μV*sec)	X Value Value	Area	Calc.
1	Amoxycillin Std – 1 mcg	4821	Amoxycillin	1	1.000	24971	0.891
2	Amoxycillin Std – 1 mcg	4822	Amoxycillin	1	1.000	24852	0.886
3	Amoxycillin Std – 10 mcg	4815	Amoxycillin	2	10.000	249613	10.116
4	Amoxycillin Std – 10 mcg	4818	Amoxycillin	2	10.000	249978	10.131
5	Amoxycillin Std – 100 mcg	4819	Amoxycillin	3	100.000	2450360	100.489
6	Amoxycillin Std – 100 mcg	4820	Amoxycillin	3	100.000	2425987	99.488\

Fig.-1: *The calibration curve*

Presentation of result with adjustment for bodyweight: In order to minimize the effect of variation in weight of the volunteers, the obtained actual plasma concentration was adjusted for weight of 70 kg and then used for calculation of different pharmacokinetic parameters. Therefore, the AUC_{0-8h} and C_{max} were calculated with two values, one with the original plasma concentration of amoxicillin detected in HPLC and the other was with value obtained after adjustment for 70 kg bodyweight. The Mean Percent Absolute Bioavailability was calculated by using the adjusted values for 70 kg bodyweight.

Table I shows that there was no significant (p>0.05) difference between the subjects of two study groups in respect of age and weight.

Table II shows that in case of oral route, the C_{max} values for Bangladeshi Bangalee and Tribal healthy volunteers were 6.78 ± 1.20 & 9.10 ± 1.34 ìg/mL respectively and the difference was significant (p<0.01). The AUC_{0-8h} for Bangladeshi Bangalee and Tribal healthy volunteers were 1290.13 ± 158.39 & 1766.06 ± 188.37 ìg min/mL respectively and the difference was significant (p<0.001). However, the adjusted C_{max} values for Bangladeshi Bangalee and Tribal healthy volunteers were 5.65 ± 0.77 & 7.15 ± 0.75 ìg/mL respectively and the AUC_{0-8h} were 1036.64 ± 93.92 & 1358.04 ± 67.79 µg min/mL respectively. In case of adjusted C_{max} and AUC_{0-8h} values, the difference was highly significant (p<0.001). The T_{max} for Bangladeshi Bangalee and Tribal healthy volunteers were 82.50 **±** 32.05 & 102.86 **±** 29.28 min and the difference was not significant (p>0.05). The $T_{1/2}$ for Bangladeshi Bangalee and Tribal healthy volunteers were 96.05 **±** 3.80 & 88.15 **±** 5.33 min respectively and the difference was significant (p<0.01).

Table-I

Age and weight of the Bangladeshi Bangalee and Tribal healthy volunteers

	Bangalee (n=8) Mean ± SD	Tribal (n=7) Mean ± SD	ANOVA F	P value	t	P value
Age (in years)	23.88 ± 0.83 (23.00 - 25.00)	24.14 ± 0.69 (23.00 - 25.00)	0.45	>0.05	0.68	>0.05
Weight (in kg)	55.88 ± 3.44 (50.00 - 60.00)	54.14 ± 3.72 (50.00 - 60.00)	0.88	>0.05	0.93	>0.05

Table-II

Pharmacokinetic parameters following oral administration of 500 mg amoxicillin in Bangladeshi Bangalee and Tribal healthy volunteers

Pharmacokinetic	Bangalee (n=8) Mean ± SD	Tribal (n=7) Mean ± SD	ANOVA F	P value	t	P value
C_{max} (ìg/mL)	6.78 ± 1.20 (5.41 - 8.99)	9.10 ± 1.34 (7.63 - 11.40)	12.48	<0.01	3.50	<0.01
C_{max} (ìg/mL) *with adjusted value*	5.65 ± 0.77 (4.64 – 6.84)	7.15 ± 0.75 (6.34 - 8.15)	18.20	<0.01	4.27	<0.01
AUC_{0-8h} (ìg min/mL)	1290.13 ± 158.39 (1054.32 - 1587.21)	1766.06 ± 188.37 (1530.23 - 1975.17)	28.30	<0.01	6.06	<0.001
AUC_{0-8h} (ìg min/mL) *with adjusted value*	1036.64 ± 93.92 (873.58 - 1156.40)	1358.04 ± 67.79 (1294.27 - 1436.33)	56.13	<0.01	7.66	<0.001
T_{max} (min)	82.50 ± 32.05 (60.00 - 120.00)	102.86 ± 29.28 (60.00 - 120.00)	1.69	>0.05	1.31	>0.05
$T_{1/2}$ (min)	96.05 ± 3.80 (90.04 - 101.68)	88.15 ± 5.33 (78.84 - 94.65)	11.17	<0.01	3.26	<0.01

Table III shows that in case of intravenous route, the $C_{30\ min}$ values for Bangladeshi Bangalee and Tribal healthy volunteers were 17.88 ± 1.14 & 18.58 ± 0.71 mg/mL respectively and the difference was not significant (p>0.05). The AUC_{0-8h} for Bangladeshi Bangalee and Tribal healthy volunteers were 2297.96 ± 222.49 & 2376.41 ± 149.99 mg min/mL respectively and the difference was not significant (p>0.05). However, the adjusted $C_{30\ min}$ values for Bangladeshi Bangalee and Tribal healthy volunteers were 14.23 ± 0.30 & 14.35 ± 0.88 mg/mL respectively and the AUC_{0-8h} were 1825.34 ± 79.34 & 1831.66 ± 40.00 mg min/mL respectively. In case of adjusted $C_{30\ min}$ and AUC_{0-8h} values, the difference was not significant (p>0.05). The $T_{1/2}$ for Bangladeshi Bangalee and Tribal healthy volunteers were 97.50 ± 3.33 & 94.40 ± 2.33 min respectively and the difference was significant (p<0.05).

Adjusted value means the value obtained after adjustment of the original values for bodyweight of 70 kg. The two-way ANOVA and Student's t test was done and the level of significance was determined against appropriate degree of freedom.

Table IV shows that the Mean Percent Absolute Bioavailability in case of Bangladeshi Bangalee and Bangladeshi Tribal healthy volunteers was 56.76 ± 5.39 and 74.17 ± 3.90 respectively. The difference between these two values were highly significant (p<0.001).

Table-III

Pharmacokinetic parameters following intravenous administration of 500 mg amoxicillin in Bangladeshi Bangalee and Tribal healthy volunteers

Pharmacokinetic parameters	Bangalee (n=8) Mean ± SD	Tribal (n=7) Mean ± SD	ANOVA F	P value	t	P value
$C_{30\ min}$ (µg/mL)	17.88 ± 1.14 (16.44 - 19.38)	18.58 ± 0.71 (17.22 - 19.44)	1.93	>0.05	1.43	>0.05
$C_{30\ min}$ (µg/mL) *with adjusted value*	14.23 ± 0.30 (13.93 - 14.89)	14.35 ± 0.88 (13.05 - 15.97)	0.15	>0.05	0.07	>0.05
AUC_{0-8h} (µg min/mL)	2297.96 ± 222.49 (2012.54 - 2689.38)	2376.41 ± 149.99 (2174.85 - 2581.37)	0.62	>0.05	1.12	>0.05
AUC_{0-8h} (µg min/mL) *with adjusted value*	1825.34 ± 79.34 (1725.03 - 1959.41)	1831.66 ± 40.00 (1798.07 - 1893.75)	0.04	>0.05	0.01	>0.05
$T_{1/2}$ (min)	97.50 ± 3.33 (90.98 - 101.60)	94.40 ± 2.33 (92.39 - 98.86)	4.22	<0.05	2.10	<0.05

Table-IV

Mean Percent Absolute Bioavailability of Amoxicillin in Bangladeshi Bangalee and Tribal healthy volunteers

	Bangladeshi Bangalee			Bangladeshi Tribal			ANOVA	p value	t value	p value
	IV	Oral	MPAB	IV	Oral	MPAB	F			
Subject 1	1725.03	980.91	56.86	1798.33	1421.12	79.02	70.56	<0.01	8.43	<0.001
Subject 2	1780.29	873.58	49.07	1880.71	1432.34	76.16				
Subject 3	1917.42	1088.35	56.76	1893.75	1310.44	69.20				
Subject 4	1959.41	1156.40	59.02	1831.84	1315.23	71.80				
Subject 5	1816.51	949.33	52.26	1802.02	1296.56	71.95				
Subject 6	1753.50	1054.91	60.16	1816.89	1436.33	79.05				
Subject 7	1837.20	1107.16	60.26	1798.07	1294.27	71.98				
Subject 8	1813.39	1082.47	59.69							
Mean	1825.34	1036.64	56.76	1831.66	1358.04	74.17				
SD	79.34	93.92	5.39	40.00	67.79	3.90				

MPAB means Mean Percent Absolute Bioavailability

Calculated on the basis of adjusted AUC from plasma concentrations obtained after intravenous and oral administration of 500 mg amoxicillin and adjusted as 70 kg bodyweight

MPAB of Bangalee and Tribal were compared with two-way ANOVA and Student's t test

Figure 2 showing the time concentration curves obtained in the intravenous route. The curves reflect no difference in the shape and slope between the curves of Bangladeshi Banglaee and Tribal healthy volunteers. The C_{max} is calculated by backward extrapolation of data up to 0 minute. The C_{max} in case of Banglaee and Tribal *derived through extrapolation is 22.24 and 24.59* µg/mL respectively.

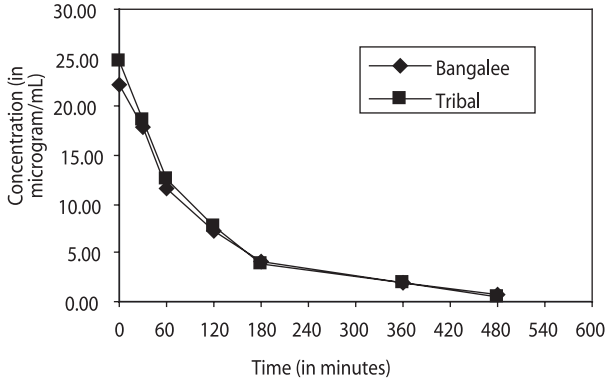


Fig.-2: *Time concentration curve obtained by plotting the mean plasma concentrations of amoxicillin estimated at different point of time (in intravenous route)*

Fig.-3 showing the time concentration curves obtained in the oral route. There are difference in the shape, slope and peak between the curves of Bangladeshi Banglaee and Tribal healthy volunteers. Difference is particularly observed in the mean concentrations obtained at 60, 120 and 180 minutes.

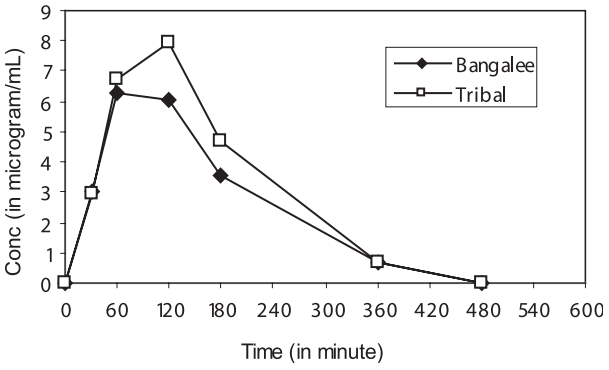


Fig.-3: *Time concentration curve obtained by plotting the mean plasma concentrations of amoxicillin estimated at different point of time (in oral route)*

DISCUSSION

Pharmacokinetic parameters of amoxicillin have been investigated at different doses of 500, 875, 1000 and 2000 mg[20-23. 25]. Previous studies using chromatography have reported C_{max} values of 8.15 µg/mL after oral administration of 500 mg amoxicillin[20], and 10.4 ìg/mL after 1000 mg amoxicillin[26] and 17.3 µg/mL after 2000 mg amoxicillin[25]. The C_{max} observed in the present study after oral administration was modestly higher in case of Tribal and lower in case of Bangalee Bangladeshi in relation to the findings of the previous studies. This feature was consistent even after adjustment of C_{max} values with bodyweight, though the reduction in standard deviation indicates certain level of correction. The variation of result with previous studies is probably due to the dissimilarity in the studied population.

The AUC_{0-8h} obtained in the present study for intravenous route both in case of Tribal as well as Banglaee was low in comparison to the result observed by Janknegt et al.1992[27], but the previous study had not mentioned any values for $C_{max.}$. The AUC_{0-8h} obtained in the present study for oral route in case of Tribal was comparable to the findings of previous studies[20,28], although the values for Bangalee was low. This feature was consistent even after adjustment of AUC_{0-8h} values with bodyweight, though the lower standard deviation indicates some correction.

Nevertheless, the T_{max} observed in the present study was parallel to the values revealed by previous researchers[20, 29] using different doses like 500 mg and 1000mg. However, the similarity in these findings reiterating the fact that different doses of the same drug have no influence on T_{max} values. The T_{max} of Bangalee volunteers was 20 minutes lower than the Tribal, though that was not significant because of high standard deviation. This finding indicates quicker absorption of amoxicillin in Bangalee volunteers, however that requires further studies with shorter interval between collection of blood sample to overcome the statistical limitations.

For $T_{1/2}$ values, different studies revealed a range of 90 minutes to 180 minutes with a mean of 100 minutes[20,27] and the present study finding is similar to those observations. The Mean Percent Absolute Bioavailability obtained in both Bangalee and Tribal volunteers in the present study appears to be lower than the previous report[30]. More importantly, the Bangalee volunteers demonstrated significantly lower bioavailability than that the Tribal volunteers, which should be explored further to relate with anthropometric parameters. This diversity might pose difficulty in understanding of population pharmacokinetics in Bangladeshi population.

A concentration of amoxicillin above the MIC for at least the 40% to 50% of time is required for an effective treatment of infection[31]. Concentration of Amoxicillin at different sites of the body usually exceeds the MIC against the common causative microbes of that infection, which is least affected by the dosing schedule[32]. MIC values of amoxicillin for *S. pneumoniae* isolates was d"2.0 µg/mL, for *H. influenzae* isolates was ≥ 0.5 µg/mL[33] and for S.

pyogenes isolates was 0.015–0.12 µg/mL[34]. Accordingly, any MIC values above these should be considered resistant for preliminary purpose unless any particular value obtained through study. The present study observed very low plasma concentrations of amoxicillin at and after 6 hours. This low concentration is whether lower than MIC for standard inoculums of some microbes or not could not be elucidated through this study. *Detail understanding about the MIC against the particular causative microbes, the required time for which plasma concentration should remain above that level and* estimation of drug by a method having lower limit of quantification at nanogram level might be helpful to explain *the clinical outcomes in any particular population.*

In order to elucidate the significant difference observed between different categories of Bangladeshi population investigated in the present study, different meta-analysis and reviews were considered. The meta-analysis and reviews revealed that metabolism of different drugs like proton pump inhibitors has little association with CYP2C19 genotype[35-36]. Study also suggests that the dose of few drugs can be increased in populations with high frequencies of HomEMs or HetEMs[37].

The revealed significant difference between two groups of Bangladeshi healthy volunteers is an interesting finding, which require further exploration with especial emphasis into genetic correlation and corresponding therapeutic outcomes. However, the extent of individual variation observed in Bangladeshi Bangalee volunteers reiterating the necessity of tailor-made drug therapy in this population. In Bangladesh, there was discussion regarding changes required in pharmacotherapy teaching and role of pharmacologist in drug regulations[38-39], which was not taken seriously in last decade. However, those pleadings and recommendations would now be strengthened by these newer observations, understandings and issues. The future research should now focus on identifying the features influencing pharmacokinetic parameters as well quantifying their role in health and disease.

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