

Reference value of serum pregnancy associated plasma protein-A (PAPP-A) in 1st trimester of pregnancy as an antenatal screening tool for genetic disorders

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ABSTRACT

Objective: Our study aims to establish the normal reference value of Serum Pregnancy Associated Plasma Protein-A (PAPP-A) in 1st trimester of pregnancy as an antenatal screening tool for genetic disorders

Material and Methods: This cross-sectional study was conducted in Chughtai Institute of Pathology, from November 2022 to April 2023. One hundred and twenty samples of healthy pregnant females between 18 – 40 years were collected by purposive, non-probability technique as per inclusion and exclusion criteria. Analysis of samples was done for quantitative measurement of serum PAPP-A levels. Statistical Analysis was done using SPSS version 21. For normality check, Kolmogorov-Smirnov test was applied with < 0.05 P value considered as significant. 2.5th and 97.5th percentiles were computed using the formula $0.025 (n+1)$ and $0.975 (n+1)$ respectively.

Results: Mean age of all pregnant female ($n=120$) subjects was 25.90 ± 4.70 years. Serum PAPP-A was measured and mean PAPP-A level for $n=120$ samples was $3539 \text{ mIU/L} \pm 2830$ with median level of 2405 mIU/L . We established the reference value in our population by Rank-based non-parametric method on the basis of 2.5th and 97.5th percentiles as $436 \text{ mIU/L} - 10000 \text{ mIU/L}$.

Conclusion: To our knowledge this is the first study to establish 1st trimester (10th to 14th week) reference value of serum PAPP-A in our population. Furthermore, our study will provide a reference for future study on establishing multiple of Median (MoM) of our population and starting an antenatal screening program by providing patient specific risk of genetic disorders.

Keywords: Pregnancy Associated Plasma Protein-A, Antenatal screening, Reference value

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INTRODUCTION

Discovered in the 1970s within the serum of pregnant women, Pregnancy-Associated Plasma Protein A (PAPP-A), also known as pappalysin-1 (EC 3.4.24.79), is an enzyme classified as a glycoprotein. It belongs to the group of metalloproteinases due to its possession of zinc-binding sites, a hallmark feature of this enzyme category [1].

Initially recognized for its abundant presence in pregnant women, PAPP-A was

originally identified as a protein primarily associated with the placenta. However, subsequent research revealed its widespread distribution across various tissues [2]. Produced by both placental syncytiotrophoblast cells and decidual cells, PAPP-A is released into the maternal circulation. It is found in a disulphide-bound form, forming a 2:2 complex with the proform of eosinophil major basic protein (proMBP). PAPP-A's primary role is to enhance the availability of Insulin-like Growth Factor (IGF) by cleaving Insulin-like Growth Factor-Binding Protein-4 (IGFBP-4). This process is crucial for ensuring normal fetal development. IGF is recognized as a growth-promoting factor essential for cell proliferation across various human tissues [3,4]. IGF is involved in movement and transport of nutrients like glucose and amino acids across placenta and it also

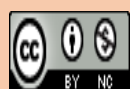
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helps in decidual trophoblastic invasion. So, the decreased levels of PAPP -A effect the IGF function which might lead to low perfusion of placenta causing various fetal growth defects [4, 5].

First-trimester screening offers the advantage of early detection and the ability to screen for various structural and genetic disorders [6]. First trimester screening is done between 11 weeks to 14 weeks of gestation [7]. Maternal and infant mortality and morbidity due to complications and disorders can be reduced if physicians utilize the antenatal screening programs and recognize abnormalities in these tests early in pregnancy [8]. Due to its significance, prenatal screening has become a fundamental component of obstetric care in numerous developed nations. This screening aims to detect fetal genetic and chromosomal abnormalities, particularly Down Syndrome (trisomy 21), utilizing serum biomarkers among other noninvasive methods [6]. The result of these serum biomarkers provides the information for making decision regarding the need of further invasive or non-invasive definitive diagnostic tests [9].

First trimester antenatal screening includes two serum markers that are Free Beta Human Chorionic Gonadotropin and Pregnancy associated Plasma Protein A (PAPP-A) [6]. Serum levels of PAPP-A increase in pregnancy with gestational age. Maximum levels are reached at birth and then the levels decline in weeks to non-pregnant levels [3]. Lower-than-normal levels of PAPP-A in maternal serum can serve as an indicator of various genetic disorders or complications associated with pregnancy [10]. The main importance of serum PAPP-A estimation is in antenatal screening of congenital disorders namely Down Syndrome which is associated with low 1st trimester levels of PAPP-A [11].

Laboratory results of screening and diagnostic tests play a crucial role in final clinical judgement and management decision by physicians. The patient test result interpretation by physicians is done by comparing the results of analytes with their population-based reference values or

reference interval (RI). These population-based reference values are established by taking appropriate reference population sample by standard methods [12]. With the establishment of reference values, Medical practitioners will use these reference values to interpret the results with assurance and accurate diagnosis [13]. The CLSI recommended guidelines are to establish reference values in local population for each analyte tested in lab [14]. If it's not feasible, IFCC recommends that reference values should be verified using analytical system or population comparison [15]. Our study aims to establish the normal reference value of PAPP-A in 1st trimester of pregnancy.

MATERIAL AND METHODS

This was a cross-sectional study, which was conducted at the Department of Clinical Chemistry & Immunology, Chughtai Institute of Pathology, Lahore, Pakistan from November 2022 till April 2023 after Institutional Review Board approval (vide reference number. CIP/IRB/1106). One Hundred and twenty Samples were collected by purposive, non-probability technique from Obstetrics and Gynecology outpatient department of Services Hospital and Moazang Hospital Lahore for the establishment of reference intervals in accordance with recommendations in CLSI guideline EP28-A3c [14.16.17]. Included samples in study were from healthy pregnant females in 1st trimester (10-14 weeks) with singleton pregnancy and normal ultrasound scan. Age group for these patients is 18 – 40 years. Samples that were excluded from the study were from pregnant females with chronic disorders, Hypertension, Diabetes Mellitus, Kidney disease, twin pregnancies, in Vitro Fertilization, previous history of genetic disorders in family and positive smoking history. Analysis of samples was done as per manufacturer instructions on fully automated immunoassay analyzer Maglumi 800 (Snibe Diagnostics) for quantitative measurement of serum PAPP-A levels. Sample rejection criteria included Hemolyzed, lipemic, and icteric samples.

Statistical analysis was done using SPSS version 21. Kolmogorov-Smirnov test was used to check the normality of the data. P value <0.05 was considered statistically significant. Reference values were calculated by Rank-based non-parametric method. Age was expressed as Mean $\pm$ SD. For serum PAPP-A levels, mean $\pm$ SD and Median values were calculated. Serum PAPP-A values of 120 samples were arranged in ascending order and as per their frequency distribution. Rank numbers were then applied according to order and frequency distribution. 2.5th and 97.5th percentiles were computed using the formula 0.025 (n+1) and 0.975 (n+1) respectively [12]. 2.5th percentile is taken as the lower reference limits and 97.5th percentile as the upper limits of the distribution of test results for the reference population [17, 18]. Furthermore, Spearman's correlation was run to determine the relationship between serum PAPP-A levels and gestational weeks. It was used as PAPP-A was not normally distributed. ANOVA test and Kruskal-Wallis test were opted to compare the Age between different groups based on Weeks and serum PAPP-A levels between different groups based on Weeks. These tests aimed to ascertain if there were statistically significant differences between these groups with P value <0.05 was considered statistically significant. ANOVA test was used as age was normally distributed. Kruskal-Wallis test was used as PAPP-A was not normally distributed.

RESULTS

All (n=120) subjects were from healthy pregnant females in 1st trimester between 10th to 14th week. Highest frequency of females 35% (42 out of 120) was in week 14. It was followed by 32.5 % (39 out of 120) in week 13, 20 % (24 out of 120) in week 12, 8.3% (10 out of 120) in week 11 and lowest frequency was in week 10 with 4.2 % (5 out of 120) (Fig 1).

In this study, mean age of all pregnant females (n=120) subjects was 25.90 $\pm$ 4.70 years. Median age was 25 years (table 1). Highest number of females 14 (11.7 %) were of 30 years of age followed by 13 females (10.5%)

of age 25 years. Lowest frequency of 0.8 % was seen in age 33, 38 and 39 years (Fig 2). Further data analysis showed mean PAPP-A level for n=120 samples was 3539 mIU/L $\pm$ 2830 with median level of 2405 mIU/L. (Table-I).

In our study, we measured serum PAPP-A in 120 samples. The data was checked for normality by Kolmogorov-Smirnov test and it showed to be following non-parametric distribution (p<0.05) in healthy pregnant females. We opted for non-parametric method (Rank-based) to establish the reference value as per CLIA guidelines [14,18]. Data was assigned ranks numbers after arranging them in ascending order (Table-II).

The lower and upper rank number were established using the formula 0.025 (n+1) and 0.975 (n+1) respectively. The 3 and 118 rank numbers were computed for 2.5th and 97.5th percentiles values. The serum PAPP-A levels corresponding to rank number 3 was the lower reference limit of 436 mIU/L and serum PAPP-A levels corresponding to rank number 118 numbers was the upper reference limit of 10000 mIU/L (Table-III).

Week wise comparison of Age with gestational weeks showed that in week 10, mean age of presenting females was 23.4 y $\pm$ 5.3. Week 11 has mean presenting age of 24.8 y $\pm$ 3.8. Furthermore, in 12th week it was 27.8 y $\pm$ 5.2, in 13th week, 26.1y $\pm$ 4.5 and in 14th week 25.5y $\pm$ 4.60. Week wise median age, age range and minimum and maximum age are shown in (Table-IV). ANOVA test was utilized to compare the age by gestational weeks and there was no statistically significant difference between and within groups with p value of 0.487 (p>0.05) and F value 0.865.

Week wise comparison of serum PAPP-A levels with gestational weeks showed that Gestational week wise median levels increase with each gestational week (Table-VI). Mean PAPP-A levels were 971 mIU/L $\pm$ 530 in 10th week, 2583 mIU/L $\pm$ 2338 in 11th week, 2559 mIU/L $\pm$ 2016 in 12th week, 3054 mIU/L $\pm$ 2335 in 13th week and 5084 mIU/L $\pm$ 3095 in 14th week. Serum PAPP-A median and range with minimum and maximum levels in each

gestational week are shown in (Table-V). Kruskal-Wallis test was utilized to compare PAPP-A levels by gestational weeks and there was statistically significant difference with p value of 0.000 ($p < 0.05$) which shows that PAPP-A values increase with every gestational week.

A Spearman's correlation was run to determine the relationship between serum

PAPP-A and gestational weeks. The results revealed a statistically significant positive correlation between these variables ($\rho = .444$, $p = .000$).

Table-I: Age and serum PAPP-A levels of healthy pregnant females presenting at 10-14 weeks (n=120).

Variable	Minimum	Maximum	Range	Mean	Median	Standard Deviation
Age (years)	18	39	21	25.90	25	4.70
Serum PAPP-A (mIU/L)	339	10000	9661	3539	2405	2830

* PAPP-A (Pregnancy-Associated Plasma Protein A)

Table-II: Frequency distribution and Ranks of PAPP-A levels in healthy pregnant females

Serum PAPP-A Level	Frequency	Rank Number
339.00	1	1
395.00	1	2
436.00	1	3
470.00	1	4
471.00	1	5
491.00	1	6
516.00	1	7
520.00	1	8
528.00	1	9
576.00	1	10
589.00	1	11
711.00	2	12-13
732.00	1	14
818.00	1	15
836.00	1	16
865.00	1	17
873.00	1	18
891.00	1	19
905.00	1	20
908.00	1	21
914.00	1	22
931.00	1	23
938.00	1	24
1032.00	1	25
1085.00	1	26
1110.00	1	27
1152.00	1	28
1165.00	1	29
1198.00	1	30
1342.00	1	31
1437.00	1	32
1466.00	1	33
1572.00	1	34
1656.00	1	35
1684.00	1	36
1708.00	1	37
1741.00	1	38
1820.00	1	39
1853.00	1	40
1855.00	1	41
1865.00	1	42
1885.00	1	43

2011.00	1	44
2022.00	1	45
2048.00	1	46
2064.00	1	47
2066.00	1	48
2076.00	1	49
2112.00	1	50
2170.00	1	51
2177.00	1	52
2192.00	1	53
2204.00	1	54
2208.00	1	55
2221.00	1	56
2327.00	1	57
2342.00	1	58
2363.00	1	59
2383.00	1	60
2428.00	1	61
2486.00	1	62
2523.00	1	63
2578.00	1	64
2704.00	1	65
2824.00	1	66
2875.00	1	67
2878.00	1	68
2895.00	1	69
2906.00	1	70
2929.00	1	71
3035.00	1	72
3194.00	1	73
3342.00	1	74
3633.00	1	75
3672.00	1	76
3732.00	1	77
3958.00	1	78
4124.00	1	79
4144.00	1	80
4357.00	1	81
4474.00	1	82
4509.00	1	83
4519.00	1	84
4550.00	1	85
4615.00	1	86
4867.00	1	87
4895.00	1	88
4910.00	1	89
4916.00	1	90
5095.00	1	91
5365.00	1	92
5585.00	1	93
5593.00	1	94
5918.00	1	95
5990.00	1	96
6283.00	1	97
6415.00	1	98
6468.00	1	99
6499.00	1	100
6513.00	1	101
6935.00	1	102
7026.00	1	103
7135.00	1	104

7669.00	1	105
7754.00	1	106
7840.00	1	107
8123.00	1	108
8288.00	1	109
8568.00	1	110
9322.00	1	111
9575.00	1	112
9694.00	1	113
9802.00	1	114
9876.00	1	115
10000.00	5	116-120

Table-III: Non-Parametric determination of serum PAPP-A reference values.

	Calculation of Rank Number based on percentile	Rank Number	Levels of PAPP-A corresponding to Rank Number (mIU/L)
Lower	0.025 (120+1) = 3.01	3	436
Upper	0.975 (120+1) = 117.97	118	10000

Table-IV: Gestational week wise comparison of age of healthy pregnant females.

Week	Variables	n=120	Minimum	Maximum	Range	Mean	Median	Std. Deviation
10	Age (years)	5	18	30	12	23.4	22.0	5.3
11	Age (years)	10	21	30	9	24.8	23.5	3.8
12	Age (years)	24	18	39	21	27.8	28.0	5.2
13	Age (years)	39	18	38	20	26.1	25.0	4.5
14	Age (years)	42	18	35	17	25.5	25.5	4.6

Table-V: Gestational week wise comparison of serum PAPP-A levels of healthy pregnant females.

Week	Variables	N =120	Minimum	Maximum	Range	Mean	Median	Std. Deviation
10	PAPP-A (mIU/L)	5	471	1865	1394	971	873	530
11	PAPP-A (mIU/L)	10	470	7135	6665	2583	1631	2338
12	PAPP-A (mIU/L)	24	339	10000	9661	2559	2016	2359
13	PAPP-A (mIU/L)	39	436	8288	7852	3054	2342	2335
14	PAPP-A (mIU/L)	42	576	10000	9424	5084	4331	3095

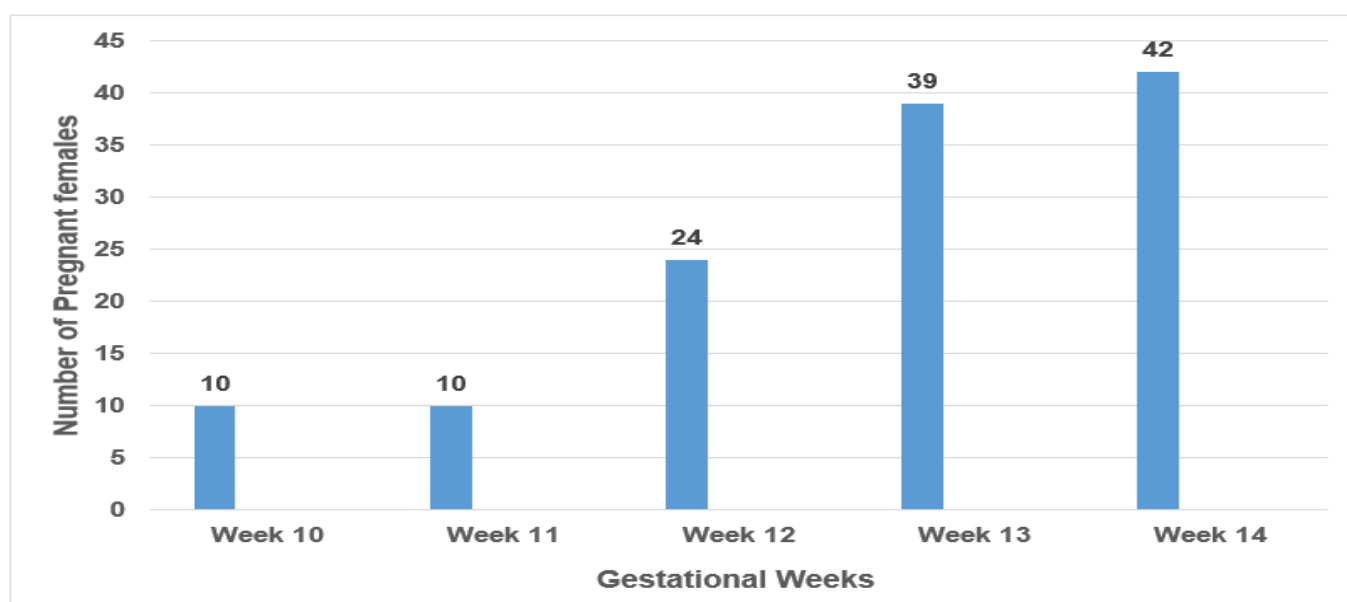


Figure-I: Frequency distribution of Healthy Pregnant females in gestational weeks

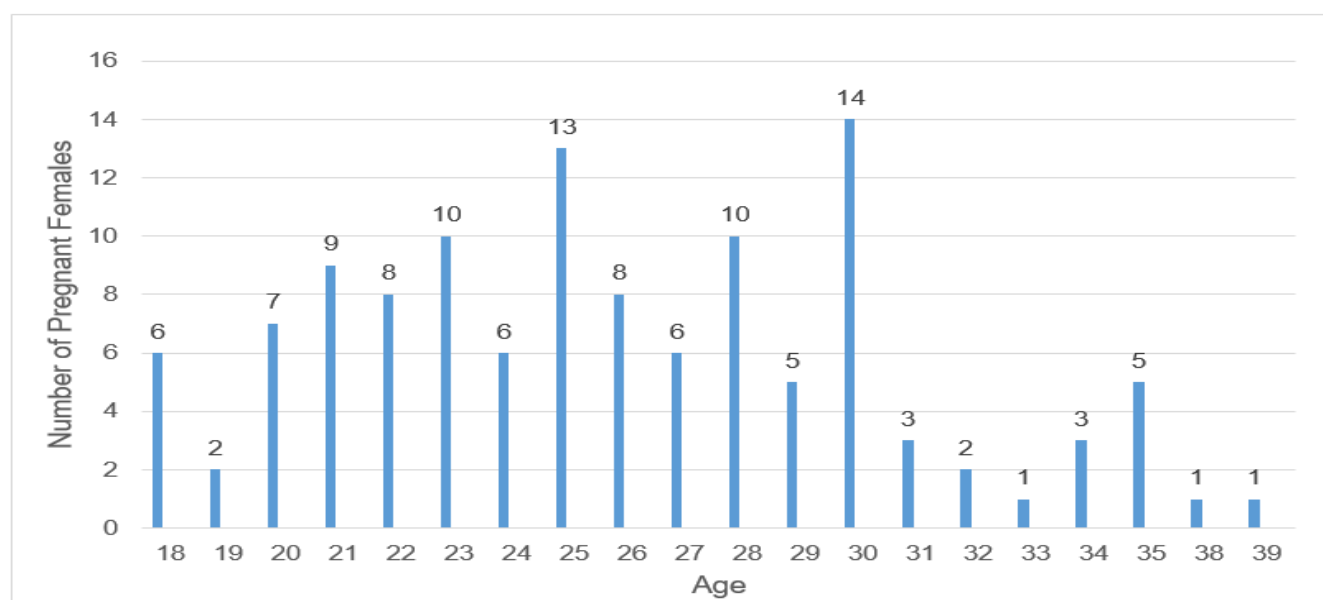


Figure-II: Frequency distribution of healthy pregnant females with age.

DISCUSSION

Reference values are commonly utilized by healthcare professionals, including physicians and laboratory personnel, as a crucial tool for evaluating and precisely diagnosing the health status of patients, distinguishing between those who are healthy and those who are diseased [19]. Establishing reference value of an analyte in local population is recommended [14].

Concentration of serum PAPP-A in first trimester of pregnancy has main and important role in fetal growth. The fetal growth is regulated by serum PAPP-A levels through regulation of IGF-1 [20]. Low levels of PAPP-A lead to growth retardation and further complications in pregnancies (20) (21). As previously mentioned, less than normal values of PAPP-A can also indicate various genetic disorders or pregnancy associated complications [10]. This has led us to establish the reference value of serum PAPP-A in our healthy pregnant female population.

We established the reference value in our population as 436 mIU/L - 10000 mIU/L in healthy pregnant females between 10-14 weeks. A study published in 2022 determined the median value of serum PAPP-A in Pakistani population between 9th and 12th week of gestation as 1289.43 mIU/L by ELISA method which are comparable to median levels

determined in our study between 10th and 14th week of gestation on chemiluminescence method [22]. A study done in 2020 in Iran shows normal levels of PAPP-A was considered 943–1455 mIU/L for 11–12th weeks of gestation, 1455–2243 for 12–13th weeks of gestation, and more than 2243 mIU/L for 13th weeks of gestation which are in accordance to the results in our study [23]. Another study reported that serum PAPP-A levels increase rapidly with only 3-4 days doubling time in 1st trimester of pregnancy and levels remain high till birth that corresponds to trend seen with serum PAPP-A levels in our population which show increasing mean and median levels with every passing week in 1st trimester [24]. A Japanese study was conducted for serum PAPP-A levels in 1,751 pregnant women, 11-14-week gestational values showed a range of 3.01 mIU/mL to 9.51 mIU/mL for serum PAPP-A (3010 mIU/L – 9510 mIU/L) which are comparable to our population results [25]. A study done in China established the reference values of serum PAPP-A concentration in different pregnant females in 1st trimester with mean value of 2037 mIU/L in 10th week, 2711 mIU/L in 11th week, 3582 mIU/L in 12th week, 4284 mIU/L in 13th week and 5317 mIU/L in 14th week. These results are comparable to mean serum PAPP-A levels of gestational weeks in our study which show similar trend of increasing PAPP-A levels but

slightly lower mean values as compared to the Chinese population. A study published in 2019 in Turkey showed mean serum PAPP-A levels of 3683 mIU/L $\pm$ 2486 with minimum levels of 363 mIU/L and maximum levels of 25501 mIU/L in healthy pregnant females in 1st trimester which are comparable to our results of mean PAPP-A level of 3539 mIU/L $\pm$ 2830 [26].

CONCLUSION

We established the serum PAPP-A reference value of 436 mIU/L - 10000 mIU/L in our population for 1st trimester between 10th to 14th week. As the reference values are affected with ethnic and geographical variations, every lab should establish its own reference value. Most important aspect of our study is that it will serve as a reference for further extensive study on serum PAPP-A levels in Pakistani population, calculating our populations multiple of Median (MoM) and providing patient specific risk of genetic disorder.

LIMITATIONS

This research study is for establishing the reference value of serum PAPP-A in 1st trimester of pregnancy. To calculate our population-based MoM for serum PAPP-A, more extensive studies are required for calculation of our population-based MoM and determining risk of genetic disorders.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Muhammad Masood Afzal: Main conception of the study, manuscript writing, data collection, statistical analysis, final approval of the draft

version, agree to be accountable for all aspects of the work

Muhammad Dilawar Khan, Hijab Batool:

Study design, final approval of the draft version, agree to be accountable for all aspects of the work

Tayyaba Rashid: Data collection final approval of the draft version, agree to be accountable for all aspects of the work

Akhtar Sohail Chughtai, Omar Rasheed

Chughtai: Overall supervision of the study, final approval of the draft version, agree to be accountable for all aspects of the work

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