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ORIGINAL ARTICLES

CASE REPORT

Original Article

REFERENCE INTERVAL OF PLACENTAL GROWTH FACTOR IN NON-PREGNANT AND PREGNANT FEMALES

Asma Rasheed, Muhammad Dilawar Khan, Hijab Batool, Omer Chughtai, Akhtar Sohail Chughtai, Shakeel Ashraf

Chughtai Institute of Pathology, Lahore Pakistan

ABSTRACT

Objective: To determine the reference interval of placental growth factor in healthy non-pregnant and pregnant females.

Material and Methods: This cross-sectional study was conducted at Chughtai Institute of Pathology Chemical pathology department from January 2023 – May 2023. Reference individuals were selected by purposive, non-probability sampling technique from 130 disease free non-pregnant and 130 disease free pregnant females. Informed consent was obtained from females of reproductive age group who fulfilled the inclusion criteria. Levels for PGF was measured by a fully automated immunoassay analyser Elecsys system Cobas e601. Data was analyzed by using SPSS21. Kolmogorov-Smirnov test was applied to test for normality. P value <0.05 was considered significant. The 2.5th and 97.5th percentiles were calculated at 90% CI by using the formula 0.025x(n+1) at rank number 1 and 0.975x(n+1) at rank number 7.

Results: Reference intervals were calculated by Rank-based method. Data values were arranged and rank numbers were allocated. Reference interval of PGF in non-pregnant and pregnant females were determine on the basis of 2.5th and 97.5th percentiles was 3.8 to 12.7 pg/mL and 46.43 to 1148 pg/mL respectively.

Conclusion: Current study findings add on contribution in comparison between reference interval of PGF in healthy non-pregnant and pregnant females. It will subsequently help the gynaecologist, clinicians, pathologist to interpret the results in order to decrease the feto/maternal complications related to pre-eclampsia. PGF levels can be cost effective by reducing unnecessary hospitalization and investigations in females at minimal risk of pre-eclampsia.

Keywords: Reference interval, Placental growth factor, Pre-eclampsia.

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INTRODUCTION

Pre-eclampsia (PE) is a serious complication of pregnancy which occurs in 14% [1] of Pakistani pregnant females characterized by proteinuria and hypertension around 20 weeks of gestation associated with increase fetal/ maternal morbidity and mortality [2]. PE occurs due to the release of proangiogenic factor i-e. placental growth factor (PGF) from the placenta that induces endothelial dysfunction. Moreover, levels of PGF can be used to distinguish normal pregnancies from pre-eclampsia before occurrence of clinical features [3]. In normal pregnancy, the levels of PGF steadily rises during the first two trimester and decline as the pregnancy progresses to term but in females who develop pre-eclampsia PGF levels found to be lower. PGF expression is up-regulated by hypoxiation in non-trophoblastic cells contrary to trophoblastic cells in which transcriptional activity of PGF is suppressed by hypoxia and increased by a normal oxygen environment [4].

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The PGF can be detected in healthy non-pregnant females at lower levels. Increased levels has association with cardiovascular diseases, as an indicator of pathological angiogenesis (macro and micro-vascular atherosclerosis). In addition, PGF has been shown to be an independent predictive marker of cardiovascular mortality and morbidity in patients with Diabetes Mellitus [5,6].

Reference Interval is a numerical value that helps to discriminate whether the individual is unhealthy or healthy. The reference interval for a parameter indicates a set of values for disease free group of individuals calculated on the basis of gender, age, ethnicity, pregnancy and non-pregnancy state. If certain parameter concentration falls outside reference interval, then it will be taken as a possible sign of disease and the patient will refer for further evaluation. Thus, the reference interval is considered as medical decision making and comparative tool. So, it is mandatory for diagnostic laboratories to carefully establish its own reference interval according to standard guidelines i-e. the Clinical and Laboratory Standards Institute protocols [7-10]. As per our knowledge, the reference interval of PGF has not been analyzed in our population. Present study

was designed to determine the reference interval of serum PGF levels, which is a novel biomarker for pre-eclampsia in literature. This can subsequently serve as a reference value to the clinicians and gynecologists to identify high risk and low risk females for pre-eclampsia in the local population. Moreover, this predictive biomarker can be cost effective by reducing unnecessary hospitalization, intervention and investigations in females who at low risk of pre-eclampsia [11].

MATERIAL AND METHODS

This was a cross-sectional study [11] conducted at the Chughtai Institute of Pathology in Department of Chemical Pathology, Lahore, Pakistan. It was carried out from January 2023 till May 2023 after getting approval from the Institutional Review Board (IRB) of Chughtai Institute of Pathology and Sheikh Zayed Medical Centre Lahore. Sample size was calculated in accordance with Clinical and Laboratory Standards Institute guidelines [7,12,13,14]. Reference individuals were selected by purposive, non-probability sampling technique from 130 diseased free non-pregnant and 130 disease free pregnant females with singleton fetus from 15 to 28 weeks of gestational age. Healthy non-pregnant and pregnant females were included by correlating history with medical disorders like diabetes mellitus, hypertension, autoimmune diseases, inherited disorders and by excluding any other drug history. All findings were recorded on health screening questionnaire administered before sample collection. Informed consent was obtained from females of reproductive age group (18-45 years) who fulfilled the inclusion criteria. From each participant 2ml of blood was drawn to obtain at least 1 ml of serum. Samples were centrifuged were performed at 4500 rpm for 4 minutes to obtained clear serum. According to our exclusion criteria hemolytic, lipemic and icteric samples will be rejected but during current study only one hemolytic sample was found which was rejected. PGF level was measured by a fully automated immunoassay analyzer (Elecsys system Cobas e601) based on the electrochemiluminescence methodology. The measuring range of PGF was set as 3-10000 pg/mL as no reference values were mentioned in reagent kit insert. The test PGF was performed as per manufacturer's recommendations. Before performing the test patient labeled samples which were frozen at -80°C were thawed and kept in sample rack of analyzer which incubates, mixes and makes calculation of the test value. Normal and abnormal controls were run before sample analysis. Data values were analyzed by using SPSS 21. To assess the normality of the variable Kolmogorov-Smirnov test was applied. P value <0.05 was considered statistically significant. The 2.5th and 97.5th percentiles were calculated at 90% confidence interval by using the formula 0.025x(n+1) at rank number 1 and 0.975x(n+1) which corresponded to rank number 7 [12,14].

RESULTS

Out of total 260 samples, 130 blood samples were taken from disease free non-pregnant females and 130 blood samples were from disease free pregnant females of reproductive age group from 18 to 45 years (Table-I). Ten values were manually removed from each set as an outlier by looking at the data. Histogram and normality test showed parametric distribution in non-pregnant females ($p>0.05$) (Figure-I) while non-parametric distribution ($p<0.05$) in pregnant females (Figure-II). Current study opted for non-parametric method (Rank-based method) for both non-pregnant and pregnant females as this is IFCC and CLIA recommended method for determination of reference values [12,14]. Data values were arranged in ascending order and rank number was assigned (Table-II). Reference interval of PGF in non- pregnant females and pregnant females were determined on the basis of 2.5th and 97.5th percentiles were 3.8 to 12.7 pg/mL (Table-III) and 46.43 to 1148 pg/mL (Table-IV) respectively.

Table-I: Age distribution of healthy non-pregnant and pregnant females.

Age (years)	Healthy non-pregnant females	Healthy pregnant females
18-23	75% (90)	31.7% (38)
24-30	11.7% (14)	42.5% (51)
31-35	5.8% (7)	15.8% (19)
36-40	5.8% (7)	5.8% (7)
41-45	1.7% (2)	4.2% (5)
Total 100% (n=120)	**100% (120)**	**100% (120)**

Table-II: Frequency distribution and ranks of PGF levels in healthy non-pregnant and pregnant females.

120 healthy non-pregnant females			120 healthy pregnant females		
Value	**Frequency**	**Rank No**	**Value**	**Frequency**	**Rank No**
2.67	1	1	32.99	1	1
3.00	1	2	45.08	1	2
3.80	1	3	46.43	1	3
3.92	1	4	54.50	1	4
4.30	1	5	62.18	1	5
4.81	1	6	64.88	1	6
5.00	1	7	77.17	1	7
5.04	1	8	80.98	1	8
5.12	1	9	85.29	1	9
5.13	1	10	88.80	1	10
5.15	1	11	95.12	1	11
5.17	1	12	101.60	1	12
5.23	1	13	102.00	1	13
5.26	1	14	109.70	1	14
5.32	1	15	110.40	1	15
5.39	1	16	113.50	1	16
5.61	1	17	116.50	1	17
5.83	1	18	119.60	1	18
5.85	1	19	128.00	1	19
5.88	2	20-21	131.20	1	20
6.08	1	22	132.30	1	21
6.10	1	23	143.30	1	22
6.14	1	24	144.10	1	23
6.15	1	25	144.90	1	24
6.23	2	26-27	154.20	1	25
6.27	1	28	160.80	1	26
6.34	1	29	162.10	1	27
6.59	1	30	163.60	1	28
6.66	1	31	165.60	1	29
6.75	1	32	168.80	1	30
6.81	1	33	171.70	1	31
6.84	1	34	180.00	1	32
6.94	1	35	183.40	1	33
6.95	1	36	190.50	1	34
7.06	1	37	191.20	1	35
7.11	1	38	203.80	1	36
7.14	2	39-40	204.70	2	37-38
7.15	1	41	205.90	1	39
7.18	1	42	207.10	1	40
7.20	1	43	209.20	1	41
7.39	1	44	210.70	1	42
7.45	1	45	226.00	1	43
7.48	1	46	228.00	1	44
7.50	1	47	230.60	1	45
7.57	1	48	242.70	1	46
7.60	1	49	245.90	1	47
7.71	1	50	259.40	1	48
7.72	2	51-52	261.20	1	49
7.74	2	53-54	262.90	1	50
7.82	1	55	271.10	1	51
7.83	1	56	273.60	1	52
8.00	2	57-58	287.50	1	53
8.02	1	59	291.90	1	54
8.03	1	60	298.80	1	55
8.04	1	61	300.40	1	56
8.12	1	62	301.90	1	57
8.14	1	63	303.60	1	58
8.23	2	64-65	304.90	1	59
8.30	1	66	313.40	1	60
8.41	1	67	318.10	1	61
8.44	1	68	323.50	1	62
8.46	1	69	331.00	2	63-64
8.48	1	70	332.20	1	65
8.59	1	71	338.90	2	66-67
8.70	1	72	392.00	1	68
8.74	1	73	401.10	1	69

8.83	1	74	405.20	1	70
8.84	2	75-76	408.10	1	71
8.90	1	77	417.80	1	72
9.11	1	78	420.80	1	73
9.16	1	79	424.00	1	74
9.20	1	80	426.90	1	75
9.26	1	81	430.10	1	76
9.31	1	82	446.20	1	77
9.41	1	83	448.90	1	78
9.44	1	84	457.90	2	79-80
9.48	1	85	466.60	1	81
9.52	1	86	467.70	1	82
9.59	1	87	472.60	1	83
9.69	1	88	486.10	2	84-85
9.80	1	89	487.20	1	86
9.94	2	90-91	494.70	1	87
10.02	1	92	497.50	1	88
10.08	1	93	509.60	1	89
10.17	1	94	526.00	1	90
10.20	1	95	546.10	1	91
10.30	1	96	547.50	1	92
10.56	1	97	557.60	1	93
10.74	1	98	566.30	1	94
11.00	1	99	573.00	1	95
11.02	1	100	576.00	1	96
11.07	1	101	581.30	1	97
11.10	1	102	584.30	1	98
11.14	1	103	613.20	1	99
11.18	1	104	614.10	1	100
11.29	1	105	654.10	1	101
11.30	1	106	681.70	1	102
11.34	1	107	702.80	1	103
11.39	1	108	741.90	1	104
11.41	1	109	743.60	1	105
11.47	1	110	771.80	1	106
11.74	1	111	790.50	1	107
11.75	1	112	798.80	1	108
11.77	1	113	849.00	1	109
11.91	1	114	880.40	1	110
11.92	1	115	889.70	1	111
12.02	1	116	953.30	1	112
12.73	1	117	976.70	1	113
12.91	1	118	980.70	1	114
13.08	1	119	986.70	1	115
13.08	1	120	1023.00	1	116
			1148.00	1	117
			1157.00	1	118
			1163.00	2	119-120

Table III: Non-Parametric determination of PGF reference interval in non-pregnant females.

Determination of rank number according to percentiles

Lower 0.025 x (120 + 1) = 3.025 at rank number 3.8

Upper 0.975 x (120 + 1) = 117.975 at rank number 12.73

Defining the original values which intersect with these rank numbers

Lower reference Limit: 2.5th percentile 3.8

Upper reference Limit: 97.5th percentile 12.7

Rank number and values of the 0.90 Confidence Limits of lower reference limit

Rank No1 and 7

Confidence limit 2.67 and 5.00

Rank number and values of the 0.90 Confidence Limits of upper reference limit

Rank number 120 + 1-7 = 114

Rank number 120 + 1-1 = 120

Confidence limit 11.91 and 13.08

Summary

PGF Lower Reference Limit (pg/mL) 3.8(2.67 to5.00)

PGF Upper Reference Limit (pg/mL) 12.7(11.9 to13.08)

Table-IV: Non-parametric determination of PGF reference interval in healthy pregnant females.

Determination of rank number according to percentiles
Lower 0.025x(120+1) = 3.025 rank number 46.3
Upper 0.975x(120+1) = 117.975 rank number 1148.0
Defining the original values which intersect with these rank numbers
Lower reference Limit:2.5th percentile 46.43
Upper reference Limit: 97.5th percentile 1148.0
Rank number and values of the 0.90 Confidence Limits of lower reference limit
Rank No1and7
Confidence limit 32.99 and 77.17
Rank number and values of the 0.90 Confidence Limits of upper reference limit
Rank number 120+1-7 = 114
Rank number120+1-1 = 120
Confidence limit 980.7 and 1163.0
Summary
PGF Lower Reference Limit (pg/mL) 46.43(32.99 to 77.17)
PGF Upper Reference Limit (pg/mL) 1148(980.7 to 1163.0)

Table-V: Reference values of PGF in healthy non-pregnant and pregnant females

	Percentile PGF assay (pg/mL)	
	Non-pregnant healthy females	**Pregnant healthy females (15 to 28 weeks of gestation)**
5th percentile	4.8 pg/mL	65.4 pg/mL
50th percentile	8.0 pg/mL	315.7 pg/mL
95th percentile	11.9 pg/mL	986.4 pg/mL

Table-VI: Comparison of reference values of PGF (pg/mL) in different studies

	Verlohren S *et al*	**Craig Saffer *et al***	**Current study**
5th Percentile	15-20 weeks	20-23 weeks	15-28weeks
	66.2 pg/mL	76.0 pg/mL	65.4 pg/mL
50th Percentile	20-23 weeks	24-28 weeks	15-28weeks
	264.0 pg/mL	417.0 pg/mL	315.7 pg/mL
95th Percentile	24-29 weeks	24-28 weeks	15-28weeks
	1117.0 pg/mL	1181.0 pg/mL	986.4 pg/mL
Instrument	ECL/ Elecsys cobas e 601	Triage PGF test (Alere, San Diego, USA) P-O-C fluorescence immunoassay	ECL/ Elecsys cobas e 601

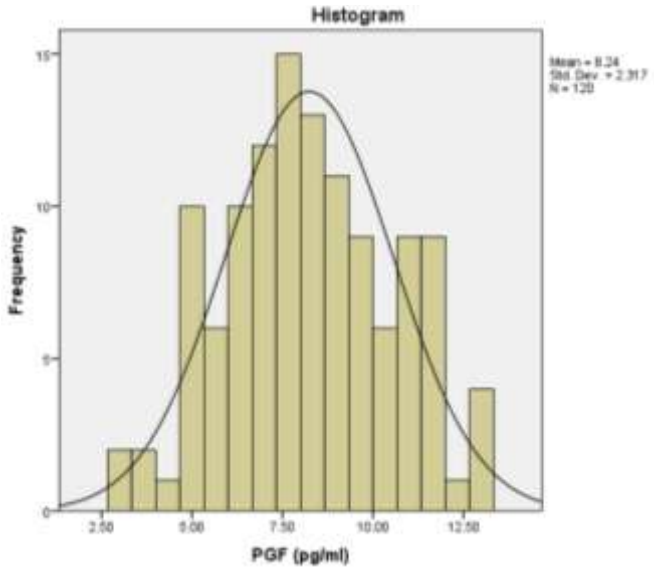

Figure-I: Histogram of PGF level (pg/mL) in healthy non-pregnant females

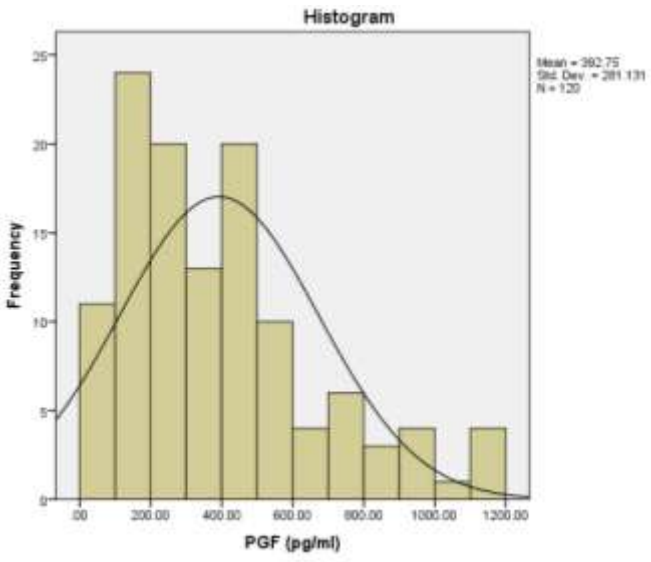

Figure-II: Histogram of PGF levels (pg/mL) in healthy pregnant females

DISCUSSION

Reference intervals/values are the most common decision-making tool used to interpret pathology reports. Different screening models use low levels of PGF as a predictive biomarker of early onset pre-eclampsia during pregnancy. Also, there is association of increased levels of PGF with cardiovascular diseases in non-pregnant females [15] but limited data is available for establishment of reference values of PGF especially in local population. The current study aimed to determine reference interval for PGF in healthy non-pregnant and pregnant females in Pakistani population.

A Prospective Multicenter Study was conducted by Verlohren S *et al* to define the reference interval of PGF for normal pregnancies in which 877 pregnant females(normotensive) from Europe (Germany, Austria, Spain, Switzerland, Czech Republic) were included. All females had a singleton, uneventful/uncomplicated pregnancy (i.e. no IUGR, no preeclampsia or HELLP syndrome). Levels of PGF were determined by Elecsys PGF assay. They reported the reference values of PGF according to gestational age (15 to 28 weeks) at 5th percentile 66.2, at 50th percentile 264 and at 95th percentile 1117 [16]. These findings correspond to current research finding (Table-VI).

Another longitudinal multicenter study was conducted in North America by Craig Saffer *et al* on establishment of reference values and cut-off for PGF in normotensive females without signs and symptoms of preeclampsia. Non-parametric centiles of distribution of PGF were used. From 247 females serial blood samples were collected in different gestational age intervals from 20–24, 25–29, 30–32, 33–35, 36–37 and 38–40 weeks). The 5th centile of PGF was 76.4, 141.1, 139.3, 65.5, 31.7, and 23.4 pg/mL in each respective gestational age interval[17]. The findings of this study also support current study findings (Table-VI).

Kelsey *et al* conducted a study in Canada who included 979 high risk pregnant females with a singleton alive fetus underwent PGF testing in between 20 to 36 weeks of gestation. A single value of PGF of 100 pg/mL was used as the cut-off value to classify females as having normal PGF levels (≥100 pg/mL) or low PGF levels (<100 pg/mL). Out of 979 pregnant females, 374 with a normotensive pregnancy outcome had PGF level >100pg/mL while 244 females who developed gestational hypertension, 189 females developed late onset pre-eclampsia, 172 females developed early onset pre-eclampsia had PGF levels <100pg/mL. These findings also support the importance of current study which emphasis on establishment of reference interval of PGF in pregnant females which can act as a predictive biomarker for pre-eclampsia [18].

During pregnancy, normal PGF levels aid in avoidance of unnecessary medical interventions and surveillance whilst low levels of PGF justify the provision of vigilant feto-maternal care and added interventions like optimally and timely administration of steroids for fetal lungs maturity, administration of intensive care monitoring and avoidance of iatrogenic preterm birth [19]. In favor of supporting the incorporation of PGF testing in routine ante-natal investigation, the present study supported a role for PGF testing as a novel screening biomarker which can be integrated into remote communities and primary care centers. The associated risks of early onset pre-eclampsia, pre-term delivery and still-birth may warrant referral of high-risk females with low PGF levels to higher level health care centers [20].

CONCLUSION

This study established reference interval of PGF in healthy non-pregnant and pregnant females for our local population. Due to limited data availability on reference interval of PGF current study findings add on contribution in comparison between reference interval of PGF in healthy non-pregnant and pregnant females. Current study findings will help the gynecologist, clinicians, pathologist and patients to interpret the results. It can further help in decreasing maternal morbidity and mortality and fetal complications related to pre-eclampsia. Moreover, it can be cost effective for health care system by reducing unnecessary hospitalization and investigations in females at low risk of pre-eclampsia.

LIMITATION

Such studies should be conducted on large scale to get more accurate estimation of reference interval and cutoff value. More over these reference interval studies should be done in diagnosed cases of pre-eclampsia and eclampsia to see the comparison of PGF levels between healthy and diseased group.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION

Asma Rasheed: Article writing, literature search, data collection, data analysis,

Muhammad Dilawar Khan: Critical review, overall supervision of the study

Hijab Batool: Statistical analysis, proofreading

Omer Chughtai and Akhtar Sohail Chughtai: Overall supervision of the study
Shakeel Ashraf: Data collection

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Original Article

CHARACTERIZATION OF NEONATAL THROMBOCYTOPENIA AT TEACHING HOSPITAL OF RAHIM YAR KHAN

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ABSTRACT

Objective: To determine the prevalence and characterization of thrombocytopenia in neonates and the pattern of illnesses in these thrombocytopenic neonates.

Material and Methods: A cross sectional study was conducted on 400 neonates in Pathology and Pediatric departments of Sheikh Zayed Medical College/Hospital, Rahim Yar Khan, from November 2022 to July 2023. Analysis of data was done by using SPSS 23.

Results: Out of 400 studied neonates, males made up 238 (59.5%), while females were 162 (40.5%). Thrombocytopenic neonates were 76(19%) while 324(81%) neonates had normal platelet count. Out of the 76 thrombocytopenic neonates, 42(10.5%) neonates had mild thrombocytopenia, 25(6.25%) had moderate thrombocytopenia and 9 (2.25%) had severe thrombocytopenia. Degree of thrombocytopenia revealed that 46 (11.5%) of neonates had thrombocytopenia in late onset phase while 30 (7.5%) had early onset thrombocytopenia. Correlation of thrombocytopenia with several disorders displayed that neonatal sepsis and anemia were significantly linked with thrombocytopenia, followed by birth asphyxia, neonatal birth in preterm and full-term neonates, low birth weight and Neonatal jaundice, furthermore, the p-value for bleeding in neonates allied with thrombocytopenia was found significant with value of 0.03.

Conclusion: The current study discovered that mild thrombocytopenia among neonates is frequent followed by moderate and severe. Thrombocytopenia has strong correlation with neonatal sepsis, anemia, birth asphyxia, neonatal birth (pre term, full term) and bleeding. Severe thrombocytopenia can be used as a prognostic indicator in sick neonates. Hence thrombocytopenia is a frequent challenge for neonatologists to cope the serious consequences of neonatal health.

Keywords: Neonatal thrombocytopenia, Platelet, Low birth weight neonates, Sepsis, Asphyxia.

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INTRODUCTION

In the neonatal period, thrombocytopenia is a prevalent issue, particularly in critically sick and premature newborns. It is found in 1–5% babies at birth and 20–50% of seriously unwell babies [1]. In order to halt bleeding from a damaged blood vessel during normal hemostasis, biological components including platelets, blood vessels and coagulation proteins are essential. The blood carries 70-80% of platelets, with the remaining 20 to 30 percent pooling in the spleen. Normal platelet count per liter of blood ranges from 150 x 10^9 to 400 x 10^9[2]. Neonatal thrombocytopenia is characterized by a platelet count less than 150 ×10^9/L in the neonates. About 20% of newborns have severe thrombocytopenia (less than 50 ×10^9/L), whereas the majority of newborns have mild (100-150 ×10^9 /L) to moderate (50-99 ×10^9 /L) thrombocytopenia. The documented life span of a platelet is eight days [3]. A neonate born at 22 weeks or more of gestation often has platelet count within the normal range as for adults [4].

Neonatal thrombocytopenia may develop either as a result of reduced platelet production secondary to reduced megakaryopoiesis or alternatively due to increased platelet destruction or sequestration. Thrombocytopenia due to reduced platelet production in neonates occur secondary to placental insufficiency [5]. The pathophysiology of neonatal thrombocytopenia comprises of decreased platelet production, increased platelet consumption and hypersplenism [3]. The etiological factors are prematurity, birth asphyxia, low birth weight (LBW), hyperbilirubinemia, respiratory distress syndrome and sepsis [6].

Up to one-third of premature newborns hospitalized in intensive care units have thrombocytopenia. In these infants thrombocytopenia

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usually manifests in one of two ways: early-onset thrombocytopenia that sets in within 72 hours of birth, or late-onset thrombocytopenia that sets in beyond 72 hours [7]. Early onset thrombocytopenia is a mild to moderate condition that is self-limiting, typically brought on by conditions linked to placental insufficiency, such as maternal hypertension. Late-onset thrombocytopenia is usually due to the bacterial sepsis and necrotizing enterocolitis; it is often severe, prolonged and requires treatment with platelet transfusions. In full term babies, neonatal thrombocytopenia is usually severe and most commonly caused by bacterial sepsis, perinatal asphyxia and neonatal alloimmune thrombocytopenia [8].

MATERIAL AND METHODS

This cross-sectional study was conducted on 400 neonates admitted to Pediatric Ward of Sheikh Zayed Medical College/Hospital, Rahim Yar Khan from November 2022 to July 2023. Neonates were categorized into early onset thrombocytopenia (<72hours), late onset thrombocytopenia (>72hours). As per the World Health Organization (WHO), Low Birth Weight (LBW) is defined as a birth weight of less than 2500 g (up to and including 2499 grams), Pre-term: less than 259 days (37 weeks), full term: 259–293 days (37–41 weeks). Post-term: 294 days (42 weeks) or more. A full term birth has been defined as between 37 and 42 gestational weeks [9]. Convenient sampling technique was utilized to select the study subjects. Informed verbal consent was taken from the parents. Using a 95% confidence level, a prevalence of 37% for newborn thrombocytopenia, and a margin of error of less than 3%, the sample size was determined to be 259. For the purpose of increasing accuracy and reliability of the findings, the calculated sample size was inflated, and the total study subjects examined were 400. 03ml of fresh venous blood sample was drawn in EDTA containing vacutainer. Platelet count was performed in Pathology Department of Sheikh Zayed Medical College/ Hospital, Rahim Yar Khan on automated 05-part hematology analyzer BT-Pro 2300. The data was collected by using a pretested questionnaire, the variables included in questionnaire were demographic variables (age, gender and address), diagnosis of disease, and onset of thrombocytopenia (early onset and late onset). Ethical approval was taken from Institutional Review Board of Sheikh Zayed Medical College/Hospital, Rahim Yar Khan. Statistical package for the social sciences (SPSS) version 23 was used to analyze the data.

RESULTS

Out of 400 neonates, 238 (59.5%) were males and 162 (40.5%) were females. In The majority of thrombocytopenia 46 (11.5%) was diagnosed after 72 hours of age (late onset), and 30 (7.5%) before 72 hours (early onset) (Table-I). Neonates with normal reference range of platelet count had a frequency of 324 (81%), while 42 (10.5%) were mildly thrombocytopenic, 25 (6.25%) were moderately thrombocytopenic, and 9 (2.25%) were severely thrombocytopenic illustrated in (Table-II). Thrombocytopenia categorized as mild, moderate and severe according to onset showed that 16 neonates had mild, 11 had moderate and 3 had severe thrombocytopenia in early onset while in late onset of thrombocytopenia 26 were mild, 14 moderate and 6 were severely thrombocytopenic. Correlation of thrombocytopenia with several disorders revealed that neonatal sepsis and anemia has significant relationship with thrombocytopenia (P value 0.000), followed by birth asphyxia (P value 0.01), neonatal birth in preterm and full-term neonates (0.02), Low birth weight (0.12), Neonatal jaundice (0.48), furthermore, the P-value for bleeding in neonates linked with thrombocytopenia showed value of 0.03 i.e. significant (Table-III).

Table-I: Neonatal thrombocytopenia according to onset (hours).

Onset of thrombocytopenia	Frequency (%)
Normal platelet count	324 (81%)
Early onset(<72hours)	30(7.5%)
Late onset(>72hours)	46(11.5%)
Total	**400 (100%)**

Table-II: Categorization of thrombocytopenia in neonates.

Platelets counts (10^9/L)	Frequency (%)
Normal Platelet Count (>150x10^9/L)	324 (81%)
Mild Thrombocytopenia (100x10^9/L-150 x10^9/L)	42 (10.5%)
Moderate Thrombocytopenia (50 x10^9/L -<99 x10^9/L)	25 (6.25%)
Severe Thrombocytopenia (<50 x10^9/L)	9 (2.25%)

Table-III: Characterization of thrombocytopenia in relation with several disease pattern (N=400).

Diagnosis		Normal Platelets	Mild Thrombocytopenia	Moderate Thrombocytopenia	Severe Thrombocytopenia	P-value
Neonatal Birth	**Preterm**	169	29	19	5	**0.02**
	Full term	155	13	6	4	
Bleeding	**Yes**	7	38	23	8	**0.03**
	No	317	4	2	1	
LBW	**Yes**	35	32	22	7	**0.12**
	No	289	10	3	2	
Neonatal Jaundice (NNJ)	**Yes**	99	25	19	5	**0.48**
	No	225	17	6	4	
Neonatal sepsis	**Early onset**	34	16	11	3	**0.000**
	Late onset	290	26	14	6	
Birth Asphyxia	**Yes**	66	24	18	5	**0.01**
	No	258	18	7	4	

DISCUSSION

There are differences in the incidence of thrombocytopenia among neonates of different populations, depending on the community being studied. In current study, males outweighed females in number. Males had a higher incidence of thrombocytopenia (238, 59.5%) than females (162, 40.5%). Similar results were reported by Tirupathi et al. showing, 56% were males and 44% were females [10]. In the recent study, thrombocytopenia was 19% in comparison to Iran, where it was reported to be 28.5% [11]. Another study, conducted by Henry et al on 807 neonates admitted at McMaster University in Canada, discovered that 22% of the neonates were thrombocytopenic [12]. In a study by Kusamsari et al., 12% of newborns had thrombocytopenia [13]. The results of present study exhibited 30 (7.5%) patients had early onset neonatal thrombocytopenia (EOT) and 46 (11.5%) had late onset neonatal thrombocytopenia (LOT), compared with the study of Tang et al, where 65 (83%) patients had EOT and 13 (17%) patients had LOT in neonates [14]. A study on neonatal thrombocytopenia discovered that 112 infants (56%) had late-onset thrombocytopenia, while 88 (44%) had early-onset thrombocytopenia [15]. A study conducted in Nigeria reported 84.84% of the cases occurred within 72 hours (early onset) [16].

In current study, out of 400 neonates, 32 low birth weight babies significantly had mild thrombocytopenia followed by moderate in 23, and severe thrombocytopenia in 8 neonates. The results were similar to the study conducted by Khalessi et al in which 59.1% babies with thrombocytopenia were low birth weight babies [17]. Studies conducted by Charoo BA et al and Robert and Murray1 also state that neonatal thrombocytopenia was more common among low birth weight babies [18].

Current study's findings revealed preterm and full-term birth pattern correlated with mild, moderate and severe thrombocytopenia unveiling 29 preterm neonates had mild, moderate (19) and 5 with severe thrombocytopenia, while 13 full term neonates had mild, moderate (6) and 4 with severe thrombocytopenia. In an Indian study thrombocytopenia was predominantly present in preterm babies (75%) compared with term babies (25%) [10]. Similar results were reported Sri Guru Ram Dass Institute of Medical Sciences and Research, Amritsar preterm neonates developed thrombocytopenia predominantly [19]. In a study from Tehran reported mild, moderate, and severe thrombocytopenia in 43.5%, 25.8%, and 24.1% of neonates, respectively. It shows that severe thrombocytopenia is an infrequent variety [17]. Another study reported neonatal thrombocytopenia was 53.0% where mild thrombocytopenia (39.4%), (12.1% moderate thrombocytopenia and severe thrombocytopenia 1.5% of the neonates [20].

CONCLUSION

Thrombocytopenia is a common problem in the neonates, particularly those born preterm. Fortunately, most episodes are mild or moderate and resolve spontaneously without apparent clinical sequelae. Late onset neonatal thrombocytopenia i.e onset of thrombocytopenia after 72 hours was more common than early onset neonatal thrombocytopenia. Low birth weight babies were more prone to get severe thrombocytopenia.

Thrombocytopenia has strong correlation with neonatal sepsis, anemia, birth asphyxia, neonatal birth (pre term, full term) and bleeding. Severe thrombocytopenia can be used as a prognostic indicator in sick neonates.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION

Muhammad Bilal Ghafoor: Conception of study, designing, literature review and supervision of study

Faiza Sarwar: Manuscript writing, analysis and interpretation

Hafiz Muhammad Tayyab: Collection of data, literature review

Samina Waseem: Statistical analysis & critical review

Farhan Ali Khanzada: Data collection, methodology

Amna Mehmood: Data collection, experimentation

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Original Article

EMERGENCE OF CEFTAZIDIME-AVIBACTAM RESISTANCE IN *ENTERO-BACTERALES* AND *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Objective: The objective of this study is to determine the emergence of resistance to Ceftazidime-Avibactam (CAZ-AVI) by *Enterobacterales* and *Pseudomonas aeruginosa* in clinical isolates.

Material and Methods: In this cross-sectional study (6-months) March-August 2022 Carbapenem resistant *Enterobacterales* were tested for Ceftazidime-Avibactam (30/20 µg, Oxoid Pvt Ltd) using Disk diffusion technique and enzymes were identified in resistant strains by Carbapenem Inactivation Method (mCIM, eCIM) as per CLSI M100 Guidelines 2022.

Results: CAZ-AVI effectiveness has greatly decreased among *Enterobacterales* and *P. aeruginosa* isolates in recent past. Antimicrobial susceptibility testing results were interpreted using CLSI M100 document. Resistance against CAZ-AVI in *Enterobacterales* was found to be 80.8 % in *E. coli* and 72.1% in *Klebsiella pneumonia* isolates. This higher emergence is associated with CRE isolates majorly comprising MBLs in our country. Moreover, it has been observed that Metallo- β-lactamases mediated enzyme resistance is one of the major resistance patterns followed by serine carbapenemases.

Conclusion: The high frequency of resistance 77% was observed against CAZ-AVI in CRE and in CRPA, the resistance is 80.1% respectively. In our country this tremendously increase in CAZ-AVI resistance is attributed to the existence of NDM in the region.

Keywords: Carbapenem Resistant *Enterobacterales* (CRE), Metallo beta lactamases (MBLs), Carbapenem Resistant *Pseudomonas aeruginosa* (CRPA) Ceftazidime-Avibactam (CAZ-AVI), Modified Carbapenem Inactivation Method(mCIM), EDTA-modified Carbapenem Inactivation Method(eCIM), Multiple Drug Resistance (MDR)

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INTRODUCTION

Over the last few years, the prompt spread of Carbapenem resistant *Enterobacterales* has tremendously affected universal public health. Despite being a part of Human Normal flora, *Enterobacterales* can become resistant to carbapenems by acquiring certain resistant mechanisms like production of carbapenemases, cell permeability changes/ expression of efflux pumps, chemical modification of antibiotic target, and also by mobile genes on plasmids that could spread through bacterial populations [1].

Ceftazidime (CAZ) being a third-generation cephalosporin has extensive activity (cell-wall synthesis inhibitor) against gram negative bacilli. Ceftazidime-avibactam (CAZ-AVI), FDA approved (2015) is a newly combined 3rd generation cephalosporin with beta lactamase inhibitor being clinically available for only last few years. But resistance to CAZ-AVI is being observed as a serious concern. As per Global Surveillance program (Latin America) CRE isolates, 24.4% (139/570) had MBLs [2]. IDSA Guidelines (2022) recommends Ceftazidime-avibactam for infections due to CRE, while Aztreonam combined with CAZ-AVI for MBL producing CRE or Cefiderocol as a monotherapy. Knowledge about CRE isolate with specific carbapenemase production is of utmost importance towards guiding treatment decisions [3].

Avibactam (AVI) increases the antibacterial activity of CAZ against AmpC-, Extended spectrum beta lactamases (ESBL) and CRE [4]. CRE/ CRPA Spread is a crucial nosocomial issue, because only few antimicrobial agents are susceptible. Also, not many new drugs are under research to treat these pathogens. A recently published study (Pakistan, 2023) demonstrated the prevalence of CR to be 42.1% (913) in 2170 clinical isolates. The 82.2% of CR isolates were found to have Carbapenemases enzymes: NDM-1(41.1%), OXA-48(32.6%), KPC-2(5.5%), NDM-1/oxa-48(11.4%) respectively [5].

Recent increase in CAZ-AVI resistance particularly in our region, is possibly due to the presence of metallo- β-lactamases as their activity is not inhibited by avibactam [6,7]. High emergence of resistance emphasizes strict infection control to prevent spread of these organisms [8].

The main purpose of our study is to determine the emergence of resistance to Ceftazidime-Avibactam (CAZ-AVI) by *Enterobacterales* and *Pseudomonas aeruginosa* in clinical isolates.

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MATERIAL AND METHODS

A cross-sectional study of 6 months duration (March-August 2022) was done after getting IRB approval reference No CIP/IRB/1100. This study was carried out to determine the frequency of Ceftazidime-Avibactam resistance in *Enterobacterales* and *Pseudomonas aeruginosa* in Microbiology Department, Chughtai Institute of Pathology, Lahore Pakistan. Sampling was done by non-probability convenience sampling technique.

Enterobacterales and *Pseudomonas aeruginosa* isolates determined to be carbapenem resistant by standard disk diffusion method were included. All duplicate isolates of patients with Gram negative rods within one month time period were excluded.

From a total of 14,457 isolates (*Enterobacterales* and *P. aeruginosa*) identified from patient samples, 1882 were found Carbapenem resistant on routine antimicrobial susceptibility testing (AST) following the CLSI Guidelines 2022.All of the Carbapenem resistant isolates (CRE, CRPA) were further tested for CAZ-AVI Susceptibility by standard Kirby-Bauer disk diffusion method. The interpretation was made according to the given breakpoints in CLSI (For *Enterobacterales* and *P. aeruginosa*, zone diameters of inhibition S: ≥21 mm and R: ≤20 mm using 30/20 µg a disk content of CAZ-AVI) and compared with *E. coli* ATCC 35218. These plates were incubated at 37C for18-24 hours and zone of inhibition was measured. Isolates with borderline inhibitory zones (18-20) were not included in the study (requires confirmatory testing) as per CLSI Guidelines. Only 1 isolate was available of both *Citrobacter spp, Providencia spp. hence,* removed from the analysis.

CRE and CRPA isolates were then tested for enzyme identification (type of beta lactamase) through carbapenem inactivation method (CIM) where mCIM detects Carbapenemases in *Enterobacterales* and *P.aeruginosa* and eCIM together with mCIM differentiates metallo-beta lactamases from serine carbapenemases in *Enterobacterales*. Inhibitory zones of inhibition mCIM, eCIM were interpreted on plates inoculated Meropenem-susceptible indicator strain *E. coli* ATCC 25922 as mentioned in CLSI 2022 (Figure-I).

Figure-I: Identifying Carbapenemases by Carbapenemase Inactivation Method (CIM).

Final identification of the clinical isolates was done by the VITEK MS system using direct deposit from bacterial colonies in agreement with manufacturer's guidelines [9].

RESULTS

Out of the Total 1445 Carbapenem resistant *Enterobacterales (CRE)* isolates,1111 were 76.89% CAZ-AVI resistant (Figure-II). Total 780 isolates of *Klebsiella pneumoniae* were tested against CZA-AVI and 562(72.1%) were found to be resistant. Whereas resistance frequency in *Escherichia coli* 80.8%, *Enterobacter spp.* 86.8%, *Serratia marcescens* 90.6% was observed (Table-I). Allocation of the tested isolates based on gender (female 42.3%, male 57.7%) and specimen type is shown in the (Table-II).

Total 437 P. aeruginosa isolates were tested for Carbapenem (Meropenem)in which 350/437 were found resistant to CAZ-AVI with overall resistance rate of 80.1%.

Out of CAZ-AVI resistant isolates 100 were tested by mCIM and eCIM for determination of beta lactamases as per (CLSI M100 Guidelines). Among *Enterobacterales*, *Klebsiella pneumoniae* showed 93.3% MBL based resistance followed by 53.3% in E. coli, whereas Serine Carbapenemases related resistance was identified as 14.6% in E.coli isolates (Figure-III). In P. aeruginosa isolates resistance due to Metallo beta lactamases were 62.5% and Serine Carbapenemases 37.5% respectively while 31.7% of the E. coli isolates tested inconclusive for Carbapenemase detection.

Table-I: *CRE* isolates and *Pseudomonas aeruginosa* showing CAZ-AVI resistance percentage.

Group	Organism	n	CAZ-AVI Resistance (%)
Enterobacterales	*Enterobacter spp.*	53	86.8%
	Escherichia coli	527	80.8%
	Klebsiella pneumoniae	780	72.1%
	Serratia marcescens	85	90.6%
	Total	**1445**	**77%**
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	437	80.1%
	Total	**437**	**80.1%**

Table-II Showing gender, specimen-based distribution of the *CRE* isolates.

Specimen Type	Female	Male	Grand Total
Enterobacter spp.			
Blood Culture	8	16	24
Wound/PUS/Fluid	10	19	29
Escherichia coli			
Blood Culture	16	18	34
Urine C/S	190	140	330
Wound/PUS/Fluid	72	91	163
Klebsiella pneumoniae			
Blood Culture	40	80	120
NTX	7	22	29
Urine C/S	169	153	322
Wound/PUS/Fluid	122	187	309
Serratia marcescens			
Blood Culture	22	49	71
Wound/PUS/Fluid	8	6	14
Pseudomonas aeruginosa			
Blood Culture	2	8	10
Respiratory Samples	5	9	14
Urine C/S	48	151	199
Wound/PUS/Fluid	78	136	214
Grand Total	**797(42.3%)**	**1085(57.7%)**	**1882**

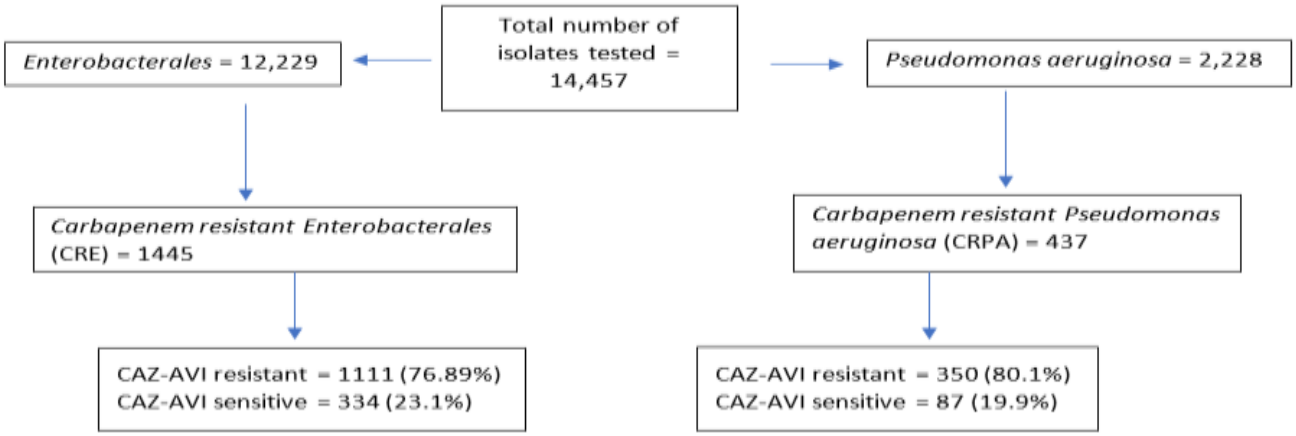

Figure-II: Flow chart showing categorization of isolates.

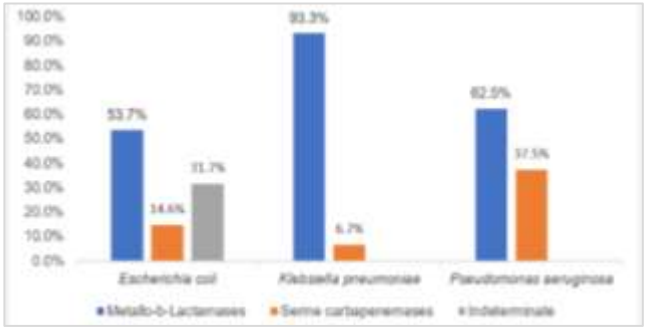

Figure-III: Enzyme based resistance (Carbapenemases) in *Enterobacterales* and *P. aeruginosa.*

DISCUSSION

Extensive drug resistance has been reported in past few decades with increased morbidity and mortality rates (CDC 2019) [10]. This study compiles the brief description of CAZ-AVI resistance in various sample. Avibactam is a non-β-lactam, β-lactamase inhibitor that acts particularly against Ambler class A, C, D beta lactamases, moreover its addition to Ceftazidime improves activity against CRE and *P. aeruginosa* [11].

Commonly reported Carbapenemases from Pakistan include NDM-1, NDM-7, VIM, IMP [5,12], NDM-1, OXA-48 [13], NDM-1, KPC-2 [14]. Carbapenem-resistant *Enterobacterales* (CRE) are micro-organisms especially resistant to carbapenems, thus difficult to treat [15]. A similar study which was done in China (2020) 103 out of total 120 CRE isolates were found to have Carbapenemases and when further CAZ-AVI susceptibility testing done on these isolates, showed 25% resistance, and all of these resistant isolates were MBL producers [16].

Even though CAZ-AVI usage is recent in Pakistan, still in our study considerable resistance pattern has been observed in *Enterobacterales* (77%), *Pseudomonas aeruginosa* (80.1%) against it.

Globally in the past few years, Clinical use of CAZ-AVI had lowered the burden to a major extent brought by XDR and MDR Gram negative bacteria. An article published in 2016 reports CAZ-AVI resistance in 30.1% of *Enterobacterales,* and this was attributed to their extended use for relatively longer times in

critically ill patients, moreover it was also suggested to further validate its effectiveness in CRE and CRPA [17]. An year later, a study published (2017) in USA shows beta lactam resistant *Pseudomonas aeruginosa* to be less susceptible (50%) to CAZ-AVI, when compared to *Enterobacteriaceae* (99%), and thus suggested CAZ-AVI, a good treatment option for beta lactam resistant gram-negative bacteria, if used cautiously [18]. As per global surveillance program (INFORM 2015-17), CRE isolates showed reduced susceptibility to CAZ-AVI, among which *E. coli* and *P. aeruginosa* were the major ones [19].

In another study which was done as part of (INFORM) global surveillance program (2014 to 2016) reports 2.3% resistance to CAZ-AVI in Colistin resistant *Enterobacteriaceae* with (MIC50) 0.25 µg/mL, and MIC of 2 µg/mL (MIC Criteria per FDA), this emphasizes on the fact that resistance has tremendously increased in recent few years of CAZ-AVI use [20]. However, in Carbapenemase resistant (CR) strains some previously done reports show marked increase in CAZ-AVI resistance reaching up to 24.7% [20,21,22]. In India, a Surveillance study was conducted on in-vitro activity of CAZ-AVI and its comparators tested against CRE (2018-2019) which showed 49% resistance to CAZ-AVI among *Klebsiella pneumonia (CRE)* isolates, and 76% in *E. coli (CRE)* isolates and 68% of these *E. coli* isolates had NDM (New Delhi Metallo-beta lactamases), whereas 24% had OXA-48 [23]. This means high resistance rates have emerged due to NDM, since last 3-4 years in India. In another study done in Iran [2020] shows that P. aeruginosa isolates from UTI patients which were resistant to CAZ-AVI, were predominantly found to have Metallo beta lactamases (75%) [24].

A study in Taiwan (Liao *et al*.,2019) has also shown resistance rate of 21% to CAZ-AVI against carbapenem resistant *P. aeruginosa* isolates in ICU admitted patients, before the drugs were officially launched and used regularly [25]. According to a meta-analysis review published in China (Li *et al*.,2021), there is no markable difference associated with CAZ-AVI either used singly or in combination in post treatment patients with carbapenem resistant Gram-negative pathogens (26). On the other hand, a study in India published 2021 which focused on clinical outcome of ICU patients on CZA, have reported 21% mortality rate with CAZ-AVI when used in combination with azithromycin, polymyxin, Fosfomycin [27].

There are few limitations of this study. Firstly, we used standard disk diffusion method for susceptibility testing, as per CLSI Guidelines (2022) however MICs could be performed along with Standard disk diffusion testing for further validation and interpretation in future. Secondly molecular characterization to identify exact underlying resistance mechanism along with epidemiological surveillance needs to be considered.

CONCLUSION

This study shows an increased resistance to CAZ-AVI in CRE (77%) possibly due to MBLs and in CRPA (80.1%) through Serine Carbapenemases and AmpC. Shortage of health care facilities and common practice of self-medication are few of the root causes of emerging antimicrobial resistance in our country. Laws and SOPs are imperative to prioritize antibiotics usage and to prevent its future critical outcomes. In this era of peak antibiotics resistance this study suggests that effective Antimicrobial Stewardship programs, infection prevention and control established in health care systems, and national action plan implementation is the need of time. All of these collectively can play significant role in combatting this problem. A high quality, multicenter study with larger sample size, based on CAZ-AVI MIC testing is suggested to confirm our findings.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION

Alina Mehwish: Substantial contributions to the conception or design of the work, acquisition, analysis, or interpretation of data for the work

Irim Iftikhar: Revision, final approval

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Original Article

ASSOCIATION OF THE PRESENCE OF BCR-ABL1 GENE REARRANGEMENTS AND MYELOID ABERRANT ANTIGENS IN PRECURSOR B-ACUTE LYMPHOBLASTIC LEUKEMIA (PRE-B-ALL) PATIENTS

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ABSTRACT

Objective: To determine the association of the presence of BCR-ABL1 gene rearrangements and myeloid aberrant antigens in precursor B-ALL patients.

Material and Methods: Both males and females, of all age groups, diagnosed with precursor B-ALL on flow cytometry were included in the study. BCR-ABL1 gene rearrangement was identified by performing Fluorescence in situ hybridization (FISH) using Vysis LSI BCR/ABL, Dual color, Dual fusion translocation probe set. The results were noted and compared with the results of flow cytometry.

Results: Male patients were 36 (60%) and female patients were 24 (40%) out of total 60 patients. Median age was 22 years (range 1-73). BCR-ABL1 fusion gene was identified in 47 (78%) patients while 13 (22%) patients were negative for BCR-ABL1. Aberrant myeloid antigens were expressed in 24 (40%) patients and all of these patients were BCR-ABL1 positive. None of BCR-ABL1 negative patients expressed aberrant antigens. There was statistically significant association between BCR-ABL1 positivity and CD117 expression ($P<0.05$).

Conclusion: In developing countries like Pakistan where specialized investigations like cytogenetics are not easily available, the aberrant phenotype can be used as a screening test for predicting the cytogenetics with poor prognosis like BCR-ABL1.

Keywords: BCR-ABL1, Pre-B-ALL, Myeloid aberrant antigens, Flow cytometry, FISH.

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INTRODUCTION

Acute lymphoblastic leukemia is a clonal hematopoietic disorder that involves the malignant proliferation and accumulation of immature lymphoid cells called lymphoblasts in the peripheral blood, bone marrow and other organs [1]. It is one of the most common childhood malignancies, occurs mainly in children and accounting for more than 20% of childhood leukemias [2]. Though median age at diagnosis is reported to be ≈ 15 years, almost 20% of all leukemia cases in adults are diagnosed as ALL [3].

Based on the origin, ALL is further subdivided into two types. The one that originates from B-cell lineage is B-cell precursor ALL (Pre-B-ALL), accounting for almost 85% of the total diagnosed cases of ALL in pediatric age group and 75% of adult age group [4]. The one that originates from T-cell lineage is T-cell precursor ALL (Pre-T-ALL), that accounts for the remaining 15% of the total cases [5].

The diagnosis of ALL is assessed on the basis of 2016 WHO classification guidelines that combine the cell morphology, immunophenotypes, cytogenetics and molecular characteristics [6]. Flow cytometry is based on the principle that the normal hematopoietic and neoplastic cells express different antigen markers on their surface and cytoplasm [7]. Immunological characteristic of leukemic cells by flow cytometry helps in deciding about the different developmental stages and lineage of malignant cells. Aberrant expression of antigens means abnormal expression of cell specific lineage antigens which is not normally associated with acute leukemia of that specific lineage [8]. Different genetic defects can result in aberrant phenotype expression, that in turn is associated with unfavorable outcome.

A number of genetic alterations and chromosomal abnormalities can occur in Pre-B-ALL, that forms the basis for the updated WHO classification of B-ALL into 2 main classes: B-ALL with recurrent genetic abnormalities and B-ALL not otherwise specified [9]. Among recurrent genetic abnormalities, Philadelphia chromosome t (9;22) with the resultant BCR-ABL1 fusion gene is the most common cytogenetic abnormality associated with Pre-B-ALL [10]. Philadelphia positive pre-B-ALL is an

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aggressive disease with poor prognosis due to treatment resistance and hence a shorter survival rate. However, the prognosis of Philadelphia positive ALL patients has been significantly improved with the introduction of tyrosine kinase inhibitors (TKIs). There have been studies that showed the possible association between BCR-ABL1 positivity and aberrant myeloid antigen expression in patients of Pre-B-ALL [11,12,13].

In a developing country like Pakistan, little data is available about the incidence of leukemia due to lack of national cancer registry. A collective cancer registry report by Shaukat Khanum memorial cancer hospital and research centre [12] showed that ALL is among the three most common childhood malignancies with 2322 (19.4%) cases of ALL out of total 11943 reported cases from Dec,1994 to Dec, 2022. In a study by Rifat et. Al [14], the results of the Global Cancer Project, conducted in the year 2020 and accessible on https://gco.iarc.fr, showed that leukemia has the highest incidence (4.3 in 1000000) and highest mortality rate (3.4 in 1000000) in Pakistan among South Asian countries.

This high mortality rate may be attributable to the lack of proper diagnostic facilities and timely identification of poor prognostic factors. Though WHO guidelines recommend BCR-ABL1 testing of all the ALL patients for risk stratification, there are only a limited number of tertiary care centers in Pakistan who are following this protocol. This is probably due to high cost and unavailability of cytogenetics at these centers. Our study aims to determine the association of the presence of BCR-ABL1 gene rearrangements and myeloid aberrant antigens in precursor B-ALL patients. Early suspicion about BCR-ABL1 positivity can be made in ALL patients who show aberrant myeloid antigens. This can help in taking decision regarding the addition of TKIs to the routine chemotherapy and in predicting the prognosis of the patients.

MATERIAL AND METHODS

It was a cross sectional analytical study that was conducted at Chughtai institute of Pathology from March 2022 to August 2022. Approval was obtained from the ethical and research committee of the institute. The sample size was calculated by using cochran provided formula that allows us to calculate the ideal minimum sample size from unknown population with a desired confidence level (Z), level of precision (e) and estimated proportion of the disease in the population. In this study, by reviewing the systematic literature [12] and expert opinion, the proportion of ALL in our population is 19% with e=8% and 90% confidence level. By using this value in formula, the minimum sample size of 60 was obtained. Total 60 untreated patients, both males and females, of all age groups, newly diagnosed with precursor B-ALL on fluorescence activated cell sorting (FACS) flow cytometry using BD FACS Lyric analyser, were included in the study. Informed consent was taken from all the patients. Immunophenotypic findings of these patients on flow cytometry were entered in a data sheet. 5ml of peripheral blood sample was taken from each patient in sodium heparin tube following standard procedures. Vysis LSI BCR/ABL, Dual color, Dual fusion translocation probe set was used for the identification of BCR-ABL1 gene rearrangement by FISH. The results were noted and entered in data sheet. Patients with types of leukemia other than ALL, already diagnosed CML patients in blast crisis, patients on chemotherapy and relapsed cases of leukemia were excluded from the study.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-21) for data analysis (SPSS Inc., Chicago, IL, USA). Frequencies and percentages were calculated for categorical variables and mean, median and standard deviation for quantitative variables. Based on the BCR-ABL1 positivity, two groups were created: BCR-ABL1 positive cases and BCR-ABL1 negative cases. Data was checked for normality using Shapiro wilk test. The Fisher's exact tests were used to assess the statistical significance of the different observations between the above-mentioned groups. P value of <0.05 was considered statistically significant.

RESULTS

Out of total 60 patients, 36 (60%) were males and 24 (40%) were females. We included both pediatric and adult patients in our study. Median age was 22 years (range 1-73). BCR-ABL1 fusion gene was identified in 47 (78%) patients while 13 (22%) patients were negative for BCR-ABL1 (Figure-I).

Based on BCR-ABL1 positivity (Figure-III), patients were divided into two groups: BCR-ABL1 positive group and BCR-ABL1 negative group. Immunophenotype results of both groups were noted and compared using Fisher's exact tests. The expression of antigens is measured qualitatively as positive or negative. Comparison of different antigen positivity between BCR-ABL1 positive and BCR-ABL1 negative cases is shown in Table-I.

In the BCR-ABL1 negative group, the most frequently expressed antigens were Tdt, CD79a and CD10 which are present in all the cases (100%), followed by CD19 (92.3%), HLADR and CD34 (both 69.2%) and CD20 (53.8%). None of the cases showed aberrant myeloid antigen expression i.e. CD117, MPO and CD13. In the BCR-ABL1 positive group, again the most frequently expressed antigens were Tdt and CD10 (both 97.8%), followed by CD79a (95.7%), HLADR (91.4%), CD19 (89.3%) and CD34 (80.8%). In contrast to BCR-ABL1 negative group, this group showed significant aberrant myeloid antigen expression (Figure-II) i.e. CD117 positivity in 17 out of 47 cases (36.1%) with a P value of 0.012, and MPO positivity in 5 cases (10.6%) and CD13 positivity in 2 cases only (4.2%) with a P value of 0.575 and 1.000, respectively.

Table-I: Frequency of different markers in BCR-ABL1 positive group and BCR-ABL1 negative group and their comparison with each other. The statistically significant difference of MPO positivity shown in red.

Markers	BCR-ABL1 positive group	BCR-ABL1 negative group	P value
Tdt	46/47 (97.8%)	13/13 (100%)	1.000
CD34	38/47 (80.8%)	9/13 (69.2%)	0.450
CD19	42/47 (89.3%)	12/13 (92.3%)	1.000
CD20	30/47 (63.8%)	7/13 (53.8%)	0.535
CD79a	45/47 (95.7%)	13/13 (100%)	1.000
CD10	46/47 (97.8%)	13/13 (100%)	1.000
CD117	17/47 (36.1%)	0/13 (0%)	**0.012***
MPO	5/47 (10.6%)	0/13 (0%)	0.575
CD13	2/47 (4.2%)	0/13 (0%)	1.000
HLADR	43/47 (91.4%)	9/13 (69.2%)	0.059

Table-II: Comparison of incidence of BCR-ABL1 in ALL patients in Pakistan and other countries.

Authors [Ref.]	Total number of cases	t (9;22) (BCR-ABL1)	Country
Haider *et al*.[17]	**623**	**16.8%**	**Pakistan**
Raza *et al*.[18]	**41**	**85.4%**	**Pakistan**
Iftekhar *et al*.[19]	**150**	**10%**	**Pakistan**
Magatha *et al*.[20]	**84**	**4.8%**	**India**
Velizarova *et al*.[21]	**30**	**17%**	**Turkey**
Carranza *et al*.[22]	**143**	**7%**	**Guatemala**
Azam *et al*.[23]	**38**	**35.7**	**Bangladesh**
Present study	**60**	**78%**	**Pakistan**

Figure-I: Frequency of BCR-ABL1 fusion gene in Pre-B-ALL patients.

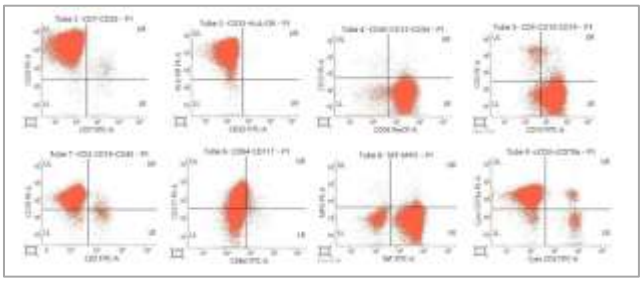

Figure-II: Immunophenotypic results by flow cytometry in a patient with Pre-B-ALL harboring the BCR-ABL1 fusion gene and expressing aberrant myeloid antigen (CD117).

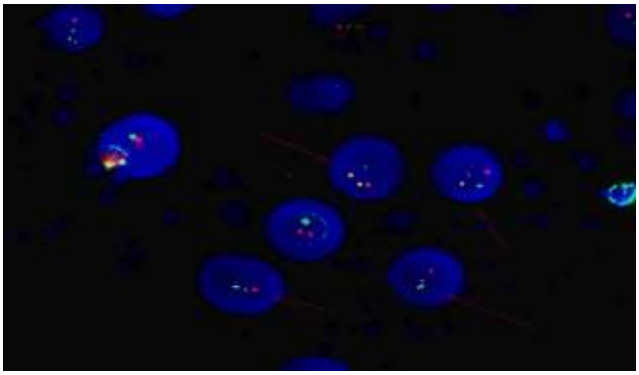

Figure-III: FISH analysis in a Pre-B-ALL patient showing BCR-ABL1 fusion gene. Performed by using Vysis LSI BCR/ABL, Dual Color, Dual fusion translocation probe set: ABL (Red signal), BCR (Green signal), Fusion (Yellow signal) indicated by arrows.

DISCUSSION

In this study, we focused on the evaluation of different cell surface antigens, including aberrant markers, expressed by blast cells in known cases of Pre-B-ALL by flow cytometry and their association with BCR-ABL1 positivity. Many studies have reported the frequency of aberrant myeloid antigens in patients of Pre-B-ALL. In a study by Rezaei *et al*. [1], the most frequently expressed markers were CD19 (100%), HLADR (98.6%), CD79a (96.5%) and Tdt (86.2%). Aberrant myeloid antigens were detected in 7 out of 89 patients of B-ALL (10.1%), out of which CD13 was the most frequently expressed aberrant marker (5.8%), followed by CD33 (2.9%) and CD117 (1.7%). None of the patient expressed MPO. Likewise in our study, the most frequently expressed markers were CD10 (100%) and Tdt (100%), followed by CD79a (95.7%), HLADR (91%) and CD19 (89.3%). In contrast, aberrant myeloid antigen positivity in our study was 36.1% with CD117 being the most frequently expressed aberrant marker (36.1%).

Venugopalan *et al*. [16] investigated aberrant immunophenotype expressions in leukemia patients. In their study, 25% (11/44) of B-ALL patients

expressed aberrant immunophenotypes and the most common aberrancy detected was expression of CD33 (13.6%), followed by CD13 and CD14 expression in 9.1% cases. These results are in contrast to our study as the most commonly expressed aberrant myeloid marker was CD117 (36.1%) in our study.

Cytogenetics have got therapeutic as well as prognostic significance while managing acute leukemias. A number of recurrent genetic abnormalities are found to be associated with B-ALL patients, most common being t (9;22) (BCR-ABL1) with a global incidence of 20-30% [12]. We compared the frequency of BCR-ABL1 fusion gene in B-ALL patients as reported by different studies in Pakistan and other countries in Table-II.

We reported a high incidence (78%) of BCR-ABL1 in Pre-B-ALL patients in our study i.e. comparatively more than that reported in literature. Our results were similar to the results of another study by Raza *et al*. [18], in which the reported incidence was 85.4%, while our results were in contrast to few other studies conducted in Bangladesh [23], Pakistan [17,19], Turkey [21], Guatemala [22] and India [20] which reported relatively lower incidence.

There are a number of studies that reported the association between aberrant myeloid antigen expression and the BCR-ABL1 fusion gene in patients of Pre-B-ALL. Gupta *et al*. [11] used Multiplex RT-PCR assays for detection of recurrent genetic abnormalities and found them in 38.36% (178/464) adult and 20.68% (108/522) pediatric BCP ALL cases. They also found that BCR-ABL1 fusion gene was seen in 31.68% (147/464) adult and 7.08% (37/522) pediatric ALL cases, whereas expression of myeloid antigens was common and observed in 29.0% of BCP-ALL patients, which expressed any one of the myeloid antigens as CD13 [Adults: 47/147 (31.97%), Pediatric: 8/37 (21.62%) P<0.0001], CD33 [Adults: 50/147 (34.01%), Pediatric: 7/37 (18.91%) P<0.0001], and CD117 [Adults: 4/147 (2.72%), Pediatric: 2/37 (5.40%) Statistically not significant]. In comparison, in our study sample size was small and we reported statistically significant association between CD117 expression and BCR-ABL1 positivity (P<0.05) while no association between BCR-ABL1 fusion gene and expression of MPO (P=0.575) and CD13 (P=1.000).

In a study by Tong *et al*. [24], it was demonstrated that B-ALL constitute 78.2% of the total 110 adult patients with ALL. The most common cytogenetic abnormality among 73 patients subjected to karyotype was the Philadelphia (Ph) chromosome, which was found in 23.3% (17/73) of the total cases and 28.8% (17/59) of B-ALL patients. This incidence was lower than reported in our study mainly due to the fact that they analyzed the cases only by routine conventional karyotyping while we used FISH for BCR-ABL1 fusion gene analysis. Myeloid antigen expression was found in 47.3% of the 110 adult ALL cases analyzed and CD13 was the most commonly expressed aberrant antigen in ALL patients (32.1 %). But in contrast to our study, there was no-statistically significant difference observed for expression of aberrant myeloid antigens in Philadelphia positive and Philadelphia negative Pre-B-ALL.

There are many studies conducted in Pakistan that assessed the incidence of BCR-ABL1 in ALL patients and many that described aberrant antigens expression in leukemia patients. In a study by Jawad *et al*. [25], aberrant myeloid expression was observed in 13 (17.8%) out of 73 cases of ALL. Among these, CD13 (11%) and CD33 (12.3%) were most frequently expressed. This is in contrast to our study as 17 out of 47 cases (36.1%) in our study expressed CD117 while only 2 cases (4.2%) expressed CD13. Our results were also in contrast to another study by Shahni *et al* [26] which studied aberrant phenotype in ALL patients and observed this only in seven out of 71 ALL patients. Most frequently expressed aberrant marker in ALL was CD13 and CD33. Also, these were expressed only in T-ALL (CD13, CD33 and HLA-DR) while 2% cases of B-ALL showed co-expression markers of T-cell (CD7) and myeloid origin (CD13 and CD33).

Reported incidence of BCR-ABL1 in Pre-B-ALL patients from Pakistan is lower [27,28], 16-16.8% in both studies, as compared to the incidence reported in our study (78%). Raza *et al* [29] reported high incidence (85.4%) of BCR-ABL1 almost similar to our study.

There is very limited data available that could identify the association between aberrant myeloid antigen expression and BCR-ABL1 fusion gene in Pre-B-ALL patients. Our study is the first in Pakistan that assessed the association between these two and found a statistically significant association between expression of CD117 and BCR-ABL1 fusion gene in Pre-B ALL patients.

CONCLUSION

A hematopathologist should be aware of the aberrant antigens expression while reporting a case of Pre-B-ALL by flow cytometry. This aberrant phenotype can be used as a screening test for predicting the cytogenetics with poorer prognosis like BCR-ABL1 in these patients. In a developing country like Pakistan

where specialized investigations like cytogenetics are not available in all the centers, this can help in assessing the prognosis as well as in managing the patients with targeted therapies like TKIs in addition to chemotherapy.

LIMITATIONS OF THE STUDY

The study was done at a single centre and sample size was also small. Sample size was limited due to cost burden. Larger cohorts with large sample size should be conducted at multiple centers.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION

Ayesha Younas: Literature search, data collection, statistical analysis and article writing

Isma Imtiaz: Literature search and data collection

Mohammad Jamil Awan: Sample analysis by FISH.

Ayisha Imran: Drafted the study design and proof reading

Touqeer Nazir: Data collection and sample collection

Noman Aslam Malik: Overall supervision of the study

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Original Article

INCIDENCE OF VULVO VAGINAL CANDIDIASIS IN YOUNG WOMEN; EXPERIENCE FROM A TERTIARY CARE HOSPITAL, KARACHI PAKISTAN

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ABSTRACT

Objective: To study the incidence of *Candida* species in women of child bearing age presenting with abnormal vaginal discharge with and without symptom of pruritus vulvae.

Material and Methods: The descriptive cross sectional study was done from Feb 2022 to Jan 2023, HVS of 300 women were taken who presented with abnormal discharge from vagina and pruritus vulvae. Samples were taken for microbiological culture testing, after thorough assessment of pertinent gynecological and obstetric history. The cultures were followed up and the results recorded.

Results: The most frequently identified organism causing abnormal vaginal discharge was *Candida albicans* (62.27%) with pregnant women experiencing a 59.09% considerably higher prevalence rate. The study found that vaginal candidiasis symptoms were itching, white curdy vaginal discharge, burning, and dyspareunia. *Candida albicans* was found in 62.2% of cases, while non-albicans were found in 37.7%. Pregnant women accounted for 59.09% of positive cases, with 74 cases of *Candida* albicans and 56 cases of non-albicans. Non-pregnant women accounted for 40.9% of positive cases, with 63 cases of *Candida albicans* and 27 cases of non-*albicans*.

Conclusion: Vulvo Vaginal Candidiasis associated with *C. albicans* as well as non albicans species of *Candida* is very common in women of reproductive age especially in developing countries. Specific diagnosis of is important as it leads to prompt treatment with antifungal, thus improving patient's health and preventing chronicity associated with disease.

Keywords: High vaginal swab, Vaginal candidiasis, Vaginal discharge.

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INTRODUCTION

Vulvovaginal candidiasis (VVC) is a common health issue that impacts health millions of women globally, it is estimated that around 75% women suffer from the disease at least once in their lifetime, among which approximately 50% have recurrent infection [1]. The morbidity associated with the disease and the economic loss is significant, according to an estimation approximately 138 million women experience recurrent vulvovaginitis annually [2].

VVC is defined as an inflammatory condition of the vulva and the vaginal mucosal walls, caused by overgrowth of the *Candida* species that typically live as a quiescent vaginal commensal [3]. It is the second most common cause of vaginitis after bacterial vaginosis. Asymptomatic vaginal candidiasis is difficult to determine because of the presence of *Candida* as a normal flora of vagina [4]. Vulval itching and abnormal vaginal discharge being the most common clinical symptoms [5]. Among *Candida* species *C.albicans* accounts for 85-95 % of all *Candida*l infections and non albicans species, *C.glabrata* and *C.krusei* accounting for the rest of the cases of VVC [6]. There are various factors responsible for the pathogenesis of the disease such as; hormonal fluctuations, vaginal microbiota and immune status [7]. Environmental risk factors responsible for VVC include pregnancy, obesity, diabetes, immunosuppression, frequent use of broad-spectrum antibiotics, and wearing of tightly fit clothing [8].

Diagnosis of VVC is based on clinical symptoms along with documented microbiological diagnosis which includes microscopy wet mount, Gram stain or culture of vaginal discharge especially in women who experience recurrent episodes of VVC [9], as untreated cases leads to recurrent vulvovaginal candidiasis and its sequelae. Furthermore, in pregnant women it predisposes to preterm labor, premature births, increase in urinary tract infection. While in non-pregnant women affected by recurrent vaginal candidiasis are prone

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to develop pelvic inflammatory disease and morbid sequelae like subfertility [10].

The rationale of our study is to investigate the incidence of vaginal candidiasis and the associated risks. We also aim to determine the speciation of *Candida* among women of childbearing age who present with vaginal candidiasis in our setup. This will enable us to ensure timely and effective antifungal treatment and prevent comorbidities associated with the disease.

MATERIAL AND METHODS

Our study is a descriptive cross- sectional study, conducted at a tertiary care hospital from February 2022 to January 2023, with consecutive sampling technique, carried out among women of child bearing age reporting at the Gynecology and Obstetrics Department in collaboration with Department of Pathology. Written consent was taken from all participants. All women presenting with the symptoms of vaginal discharge; including burning, itching dyspareunia, and soreness in perineum were enrolled in the study. Conversely, women who declined participation, diagnosed cases of bacterial vaginosis and patients already on antifungal therapy were excluded from the study.

A total of 300 women with the suspected *Candida* vaginitis were enrolled in study after calculating the sample size by who calculator with confidence interval 90%, population proportion 50% with margin of error 5% [11]. The recurrent VVC was defined as four or more episodes of culture-proved VVC in a year.

Local perineal and speculum examination was carried out in all symptomatic women. High vaginal swab was taken by using standardised sterile cotton-tipped wet swabs. One swab was used for direct microscopy using KOH, and the other one was applied for culture. Initial diagnosis of specimens was performed by wet mount smear under microscope showing yeast cells, gram stain, culture was applied on Sabouraud dextrose agar (SDA) (Merck, Germany), further speciation was done on CHROM agar which was used as a differential culture medium for identification of yeast species on the basis of different colors in the clinical specimen.

All the data collected from each patient was recorded in Microsoft Excel 2013, and subsequently analyzed using Statistical Package for the Social Sciences (SPSS) version 25 for evaluation using descriptive statistics. Frequencies and percentages were calculated for age, clinical details and specie differences of *Candida*.

RESULTS

A total of 300 women with the suspected *Candida* vaginitis were included in the study which was carried out at tertiary care hospital Karachi, Pakistan. Out of these, 220 patients (73.3%) were diagnosed with VVC based on culture tests (Table-I). The age of the patients ranged from 20 to 40 years with a mean age of 30 years. The most affected age group was 26-30 years with 117 cases (39%) (Table-II).

The prevalent symptoms reported were itching (21.3%), white curdy vaginal discharge (47.3%), burning (18%), and dyspareunia (13.4%). Among the positive cases, *C. albicans* was the most common species detected in 137 cases (62.2%) while non-albicans were found in 83 cases (37.7%). Out of 220 positive *Candida* cases, 90 cases (40.9%) were non-pregnant women and 130 cases (59.09%) were of pregnant women. *Candida* albicans was found in 74 cases of pregnant women while the non-albicans group comprised 21 cases of *C.glabrata,* 19 cases of *C.parasilopsis,* and 16 cases of *C.tropicalis.* In non-pregnant women, the prevalence of *Candida* species was 63 cases of *C. albicans,* 14 cases of *C. glabrata,* 7 cases of *C. parasilopsis*, and 6 cases of *C.tropicalis* (Table-III).

The study found that 21.3% of patients with pruritus vulvae had VVC, and 47.3% of cases with abnormal vaginal discharge had *Candida*-positive culture (Table-IV).

Table-I: **Prevalence of VVC & specie distribution in Pregnant and Non-Pregnant group.**

N=300		Positive Cases	*Candida albican*	Non-*Candida albican*
		220	137 (62.3%)	83
Pregnant	150	130	74(37.7%)	56
Non-Pregnant	150	90	63	27

Table-II: Distribution of VVC cases in various age groups.

Age	No of Patient	Percentage
20-25	97	31.6
26-30	117	38
31-40	86	30.4

Table-III: Isolated *Candida* species among Pregnant and non-Pregnant women.

Species	Pregnant	Non-Pregnant	%
C. Albican	74	63	62.7
C. Glabarta	21	14	15.9
C. Parasilopsis	19	7	11.8
C. Tropicalis	16	6	10

Table-IV: Characteristic feature of VVC in culture positive cases.

Symptoms	No of Patients	%	Positive for Candidiasis
Discharge	142	47.3	118
Pruritis	64	21.3	42
Both	78	26.1	60

DISCUSSION

VVC is a yeast born lower genital tract infection, frequently seen in women of reproductive age group.[12] In most of the cases VVC is diagnosed clinically by signs and symptoms without the use of specific diagnostic testing , and therefore exact etiology of infection remains unclear.[13] In our study we targeted specific groups i.e. pregnant and non-pregnant females, in order to identify the exact incidence of vaginal candidiasis in our population and to know about various *Candida* species responsible for VVC . Our study focused to estimate the incidence of VVC by specific laboratory diagnosis in patients meeting the clinical criteria. Although the incidence rate of VVC (58%), in our study was much higher as compare to other regional study Abbasi Nejat *et al* from Iran which reports (22.2 %) cases of VVC. However, our results are comparable to another study from Ethiopia by Bitew *et al* that showed 48% cases of VVC.[15]

Vulvovaginal candidiasis in the reproductive age group has been a common, disturbing concern due to its high recurrence rate. Few major factors for differences in the occurrence of VVC as mentioned in most studies are, sociodemographic characteristics.[16] In our studied group, age was taken as a potential risk factor and our findings were consistent as mentioned by other authors in literature, suggesting reproductive age group as a potential risk factor for VVC .[17]

In our studied populace etiology of vaginal candidiasis was most commonly represented by *Candida* yeasts and *C. albicans* was found to be the most (62.27%), which is also universally known to be the most common etiological agent also quoted by Pakistani studies and international studies .[18,19]

Our study showed statistically significant difference in the incidence of VVC between pregnant, (59.09%) and nonpregnant women (40.9%), findings consistent with studies carried out previously, reporting pregnant women as more vulnerable to acquire candidiasis due to reduced cell mediated immunit high estrogen levels and high glycogen levels.[20]

CONCLUSION

To conclude, VVC caused by *C. albicans* as well as non albicans species of *Candida* is quite prevalent in women of childbearing age in our region. There is also need for laboratory confirmed cases of VVC, so that the incidence of infections caused by non albicans species of *Candida* could be kept under check and appropriate antifungal therapy can be given to prevent recurrent infections.

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CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION

Ayisha Raja: Data acquisition, Data analysis, critical review, approval of the final version to be published.

Abeera Ahmed: Concept, Study design, drafting the manuscript, Literature review

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Namra Sohail Raja: Drafting the manuscript, data acquisition,

Ameera Tariq: Literature review, approval of the final version to be published

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Original Article

CORRELATION OF ER, PR, AND HER2 WITH HISTOLOGICAL GRADES OF INVASIVE DUCTAL CARCINOMA OF BREAST

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ABSTRACT

Objective: To study the correlation of histological grades of breast cancer with the expression of hormone receptors ER, PR, and HER2/neuin patients of invasive ductal carcinoma breast.

Material and Methods: A Descriptive, cross-sectional was conducted at the Department of Pathology, PNS Shifa Hospital, Karachi from 13th February 2021 to 12th August 2021.77 cases of invasive ductal carcinoma breast were included in this study with subjects falling between the age groups of 25 and 85. On H&E-stained sections, The Nottingham Scarff Bloom Richardson System variant was used to grade the histology of the samples. The formalin-fixed paraffin-embedded (FFPE) tissues were subjected to immunohistochemistry for ER, PR, and HER-2 receptors using the DAKO Envision kit.

Results: In our study, histological grading of invasive ductal carcinoma was found to be as follows; grade I in 01 (1.30%), grade II in 53 (68.83%), and grade III in 23 (29.87%) patients. Among the hormone receptors, the ER receptors revealed non-significant correlation i.e. 0.090. Also, PR receptors revealed 0.192 and HER-2 showed 0.410 values respectively in accordance to the grades of invasive breast carcinoma.

Conclusion: According to the study's findings, grade II (75.71%) and grade III (22.86%) had the highest prevalence among invasive ductal carcinoma cases. Additionally, the estrogen receptor (ER) expression was higher than that of HER-2 and the progesterone receptor (PR).

Keywords: Histologic grade, Invasive ductal carcinoma, ER, PR, HER-2.

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INTRODUCTION

Breast cancer is one of the most common cancers causing mortality in women between the ages of 45 and 55 years. It is the most frequently diagnosed cancer and the leading cause of cancer death, followed by colorectal and lung cancer for both incidence and mortality [1]. The incidence in Pakistan is 34.6%, placing it at the top position among Asian countries [2]. Factors that govern the prognosis for breast carcinoma include tumor size, lymph node involvement, histologic subtype, and histologic grade. Different subtypes of breast carcinoma were generated based on expression of ER, PR, Ki67 and HER2, positive and/or negative [3]. The human epidermal growth factor receptor-2 (HER2), progesterone receptor (PR), and estrogen receptor (ER) are frequently employed biomarkers for predictive and prognostic purposes [4]. In women with operable invasive breast cancer, the hormone receptor subtype is a significant independent prognostic predictor [5]. They support therapeutic decisions that are trustworthy and economical and are affordable substitutes for more expensive molecular subtyping techniques [6,7]. With a view to reducing mortality, it is essential to maintain efficient procedures and protocols for the early detection and screening of the disease [8,9].

The status of ER, PR, and HER2 receptors in breast cancer has become pertinent to determining prognosis in contemporary medical practice [10]. For patients with breast cancer, immunohistochemical examination of receptors like ER, PR, and HER2 is frequently done to forecast therapy outcomes [11]. The objective of the current study was to correlate ER, PR, and HER2/neu markers immuno-histochemistry status in breast cancer. Correlation with histological grading was also carried out to look at any links between these markers and the severity of the disease.

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MATERIAL AND METHODS

A Descriptive, cross-sectional was conducted at the Department of Pathology, PNS SHIFA

Hospital, Karachi from 13th February 2021 to 12th August 2021. Between the ages of 25 and 85, 77 cases of invasive ductal carcinoma, including both males and females, were included in the study. Patients below and above this age group were excluded from this study.

A sample size of 77 was calculated with the WHO calculator by taking a 95% confidence interval, 7% margin of error, and prevalence of invasive ductal carcinoma of the breast, grade I = 11% (from a Pakistani study). For this study's non-probability, consecutive sampling was used. The study includes Trucut, incisional or and excisional biopsy, mastectomy specimens diagnosed as invasive ductal carcinoma with ≥500 tumor cells. Whereas poorly fixed tissue with extensive necrosis post-chemotherapy tumors and metastatic tumors were excluded from the study. After approval from the College of Physicians and Surgeons of Pakistan and an ethical permission certificate from the Institutional Ethical Committee, the patients who fulfilled the inclusion criteria were included in the study. The formalin-fixed paraffin-embedded (FFPE) tissues were used to create slides that were stained with hematoxylin and eosin. The histologic type and grade of the tumor were determined by a microscopic examination performed by a histopathologist.

ER, PR, and HER2 status were stained using the DAKO Envision kit in accordance with the manufacturer's instructions. The Allred criteria were used to evaluate ER and PR. The HER2 status was disclosed in accordance with the ASCO/CAP recommendations.

Statistical analysis was done using SPSS version 23.0. Continuous variables including age and BMI were presented as mean ± standard deviation. Tumor grading and hormone receptors were compared by using the Chi-square test. Post-stratification Chi-square test was used. A p-value of ≤0.05 was taken as statistically significant.

RESULTS

The age range in this study was from 25 to 85 years with a mean age of 50.89 ± 14.14 years. The majority of the patients 47 (61.04%) were >55 years of age. The majority of the patients were females (97.4%). Table–I revealed Immuno histochemical analysis of the breast cancer cases with respect to estrogen receptors (ERs), progesterone receptors (PRs), and the human epidermal growth factor receptor-2 (HER2/neu). The frequencies of positively expressed cases for ER, PR & HER2 receptors are found to be 41 %, 36.2% and 51% respectively. Figures I, II &III also showed the positive immune expression of these hormone receptors.

In our study, the histological grading of invasive ductal carcinoma was found to be as follows; Grade I in 01 (1.30%), Grade II in 53 (68.83%), and Grade III in 23 (29.87%) patients. Stratification of grades with respect to ER, PR, and HER2 are shown in Tables-II, III and IV respectively, revealing the correlation of these receptor with the histological grades of invasive ductal carcinoma. The study found no significant correlation of these hormonal receptors with the histological grades of invasive breast carcinoma.

Table-I: Distribution of patients with status of receptors (n=77).

Types of Receptors	Expression	No of cases	Frequencies
ER	Positive	41	53.25
	Negative	36	46.75
PR	Positive	33	42.86
	Negative	44	57.14
HER-2	Positive	51	66.23
	Negative	26	33.77

Table-II: **Correlation of histological grade with respect to ER.**

ER	Histologic grade			p-value
	I	II	III	
Positive	01	31	09	**0.090**
Negative	00	22	14	

Table-III: Correlation of histologic grade with respect to PR.

PR	Histologic grade			p-value
	I	II	III	
Positive	01	26	06	**0.192**
Negative	00	27	17	

Table-IV: Correlation of histologic grade with respect to HER2.

HER2	Histologic grade			p-value
	I	II	III	
Positive	01	37	13	**0.410**
Negative	00	16	10	

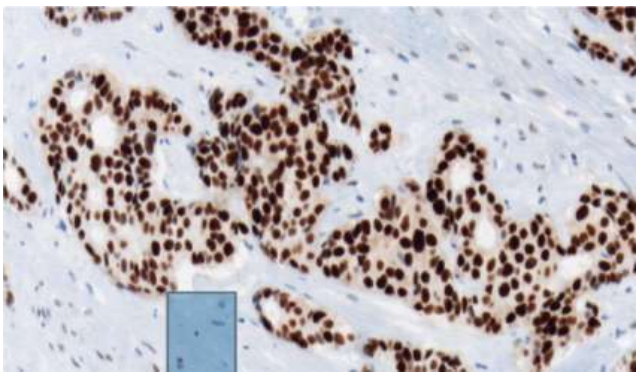

Figure-I: Estrogen receptor staining.

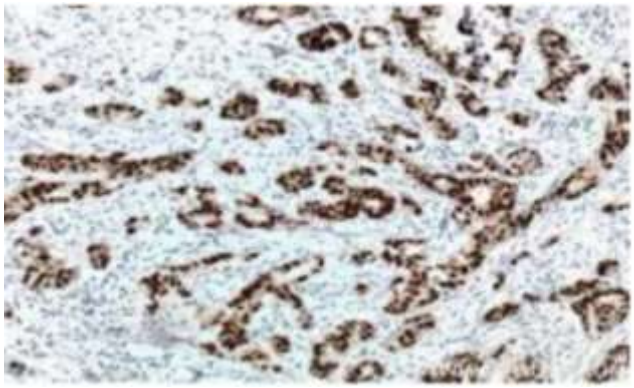

Figure-II: Progesterone receptor staining.

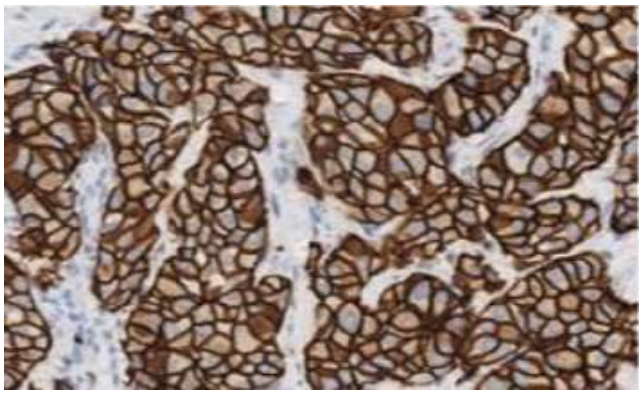

Figure-III: HER2 staining.

DISCUSSION

This study was carried out at PNS Shifa Hospital in Karachi and involved 77 patients diagnosed with invasive ductal breast cancer. The need to combine clinical pathology and cancer biology is emphasized in order to determine the optimum course of treatment and prognosis for breast cancer patients. The evaluation of hormone receptors, such as ER, PR, and HER2/neu, is frequently used to guide adjunct hormonal and targeted therapy for breast cancer patients. Better outcomes will be obtained by adopting treatment decisions on this knowledge. The ages of the participants in this study who took part in the analysis ranged from 25 to 85. The average age of the patients was calculated to be 50.89 years. The majority of the patients 47 (61.04%) were >55 years of age which is almost comparable to other studies in Pakistan [12,13].

We have conducted this study to determine the histological grading of invasive ductal carcinoma and to compare histological grades of invasive ductal carcinoma with hormone receptors in patients of invasive ductal carcinoma. In our study, histological grading of invasive ductal carcinoma was found to be as follows; grade I in 01 (1.30%), grade II in 53 (68.83%), and grade III in 23 (29.87%) patients.

In the present study the expression of ER was found in 41 (53.25%) patients, PR expression was 33 (42.86 %) and HER -2 was found to be 51(66.23%) in cases of invasive breast carcinoma.

Similar observations were made by Tummidi Santosh *et al* in a prospective study conducted at MKCG MCH Brahmapur, Odisha, India on 82 cases for a period of one year [14]. According to histological grading, ER, PR and HER-2 receptors reveals non-significant correlation. These results are partially consistent with a study [15].

Our findings are not consistent with another study conducted by Ablavi Adani in Togo (West Africa), who assessed negative ER and PR expression, and HER2 positivity. The study also revealed a significant correlation of HER-2 receptor subtype types with the stage ($p = 0.025$) and the histological grade ($p < 0.0001$) [16].

Similar observations were made by Carol A. Parise and Vincent Caggiano in the California Cancer Registry. According to the ER/PR/HER2 subtype is simple, inexpensive, easy to interpret, reliable, reproducible, and readily available for clinicians without additional tests [16]. Similar findings were made by Montemurro F Cosimo S, and Arpino G in a study [17].

Our findings are not consistent with a study conducted by Hou W, Yao Q, Niu DF, Xue WC in China, who assessed negative ER and PR expression, and HER2 positivity [18].

Our study findings co relate with a study carried out at university of Rothak where 150 samples were studied and similar expression of ER, PR and Her 2 receptors was seen in the study [19].

The most crucial element of breast cancer prognostication is histopathological grades which could be assimilated into staging systems and algorithms to choose the most appropriate treatment for patients with breast cancer [20,21].

The current study's observation of a direct association between ER, PR, and HER-2 positivity and histological grade highlights the importance of these markers as effective breast cancer prognostic tools. These results underline how crucial it is to evaluate the expression of ER, PR, and HER-2 in figuring out the tumor's grade, which in turn helps in choosing the correct treatment options. These indicators can be effective tools for management and therapy decisions. In this regard, we recommend that immune staining of the hormone receptors should be used routinely for breast cancer diagnosis as it will help in the early and timely management of high-risk patients to reduce the morbidity and mortality associated with breast carcinoma.

CONCLUSION

The histological grade is an important determinant of breast cancer prognostication and should be incorporated into staging systems and algorithms to choose the most appropriate treatment for patients with breast cancer.

STRENGTHS AND LIMITATIONS

Strengths: The main strength of this study is the fact that it establishes a direct histological correlation between the expression of hormone receptors and different histological grades of invasive ductal carcinoma breast. This significance can be employed for assessing the prognosis as well as determining the need for adjuvant chemotherapy in the patients.

Limitations: The study done at Karachi's P.N.S. Shifa Hospital admits several restrictions. First off, only patients treated at that hospital were included in the investigation of biomarkers, which may not accurately reflect the region's full population of breast cancer patients. The study's sample size was very modest, which may limit how far the results may be applied

CONFLICT OF INTEREST

None

RECOMMENDATIONS

It may be possible to gain additional knowledge and chances to examine histopathological correlations in greater detail by doing the study on a bigger sample size. Future research can improve our comprehension of breast cancer and its histological characteristics by addressing these shortcomings

AUTHORS CONTRIBUTION

Yusra Saleem: Conceived idea, study design, data collection, immunohistochemical analysis, Result interpretation,

Hona Wati: Literature review, manuscript writing. data analysis, result interpretation, proofreading & correction of entire manuscript

Beenish Hussain Nomani: Manuscript writing, data analysis, proofreading and correction of entire manuscript

Misbah Riaz: Data analysis, immunohistochemical analysis.

Shaista Bakhat: Helped to draft the manuscript, proofreading and correction

Hira Faisal: Data collection, helped to draft the manuscript, proofreading and correction

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Case Report

EXPLORATION OF HEREDITARY ANGIOEDEMA IN A YOUNG MALE WITH ACUTE ABDOMEN: EXPERIENCE FROM A TERTIARY CARE SETUP IN PAKISTAN

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ABSTRACT

Hereditary angioedema (HAE) is a freak, life-threatening genetic disorder caused by insufficiency or impaired function of the C1 inhibitor. HAE is difficult to diagnose due to its rarity, similarity of symptoms with other conditions, and lack of diagnostic facilities for C1 inhibitor in tertiary care centers. This case is being reported due to its unusual presentation with symptoms mostly affecting the gastrointestinal tract.

Case of a young male who presented with abdominal pain and dysentery. History revealed its periodic nature since puberty, only abdominal tenderness was positive on clinical examination. Blood complete picture, ESR, CRP, Stool investigations for WBCs, ova/parasites, occult blood, stool culture, *Clostridium difficile* toxin and antigen were also negative. CT scan of abdomen revealed edema of bowel wall and acute extensive colitis involving the ascending and transverse colon. Complement C4 levels <0.05 g/l and very low values of C1 esterase inhibitor <0.2 mg/dl led to diagnosis of HAE type I.

Keywords: Abdominal pain, Complement levels, C1 inhibitor, Hereditary angioedema.

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INTRODUCTION

Hereditary angioedema is mainly an autosomal dominant disorder but also results from de-novo mutations in 20 to 25% of cases. There are no racial or gender disparities in its occurrence, and incidence varies between 1:50,000 and 1:150,000 [1]. This potentially life-threatening disease is clinically distinguished by intermittent episodes of cutaneous angioedema (non-pruritic and asymmetric without urticaria and submucosal swelling with severe abdominal pain) which are most common features but these patients can also present with genital swelling, laryngeal edema and joint swelling [2]. Laryngeal edema, is the primary cause of mortality in HAE patients as this can lead to asphyxiation. Numerous factors, such as stress, trauma, infections, surgical and dental procedures and estrogen hormone are known to cause HAE episodes [3]. HAE is categorized into three types: Type 1 HAE is caused by reduced production or lack of C1 esterase inhibitor. Type 2 HAE is characterized by normal C1-INH level with functional defects of C1-INH and type 3 HAE is associated with normal level and function of C1-INH but defect lies in the other regulators of the kinin system such as factor XII gene, plasminogen gene, angiopoietin gene, kininogen-1, myoferlin, HS3OST and HAE of unknown mutation which can only be diagnosed with genetic testing [4]. Low C1 INH levels are hallmark of acquired angioedema, which has been linked to several conditions and medications including ACE inhibitors, estrogen, connective tissue diseases, infections, leukemia, and lymphoma [5].

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CASE REPORT

A 30 year male visited the hospital emergency with sudden onset of severe pain in the left lower abdomen associated with eight episodes of non-bloody watery diarrhea in last two days. There was no history of fever, anorexia, nausea, vomiting or weight loss. No history of recent travel or sick contacts was there. His past medical history revealed that he had several similar episodes of abdominal pain since puberty that subside spontaneously over two to three days. His all siblings and mother had no significant medical history but his mother mentioned

that his father had several episodes of abdominal pain in his youth for which he used to take pain killers but had no history of angioedema on face. His father was once admitted to hospital for dyspnea but no cause was identified at that time and he never underwent laboratory investigations for HAE.

On general physical examination, he was well oriented with stable vital signs. His chest was clear, with normal heart sounds and no murmur. There was no swelling on face or any other body part. His CNS examination was unremarkable but he had slight abdominal distention, tenderness, a nd hyperactive bowel sounds. He was initially diagnosed as a case of acute abdomen and was admitted to the hospital emergency room. He was kept nil by mouth (NPO), given morphine for pain relief, intravenous fluids for hydration, and empirical I/V antibiotics including ceftriaxone and metronidazole.

Blood complete picture, ESR and CRP were within normal limits. The stool investigations for WBCs, ova/parasites, stool culture and stool for occult blood were negative. The stool for *Clostridium difficile* toxin and antigen were also negative. CT scan of abdomen revealed edema of bowel mucosa and acute extensive colitis involving the ascending and transverse colon with no evidence of perforation or obstruction. Complement levels were measured which revealed decreased complement C4 levels <0.05 g/l (Normal reference range: 0.2-0.5 g/l) and very low values of C1 esterase inhibitor <0.2 mg/dl (Normal reference range: 4-70 mg/dl). Based on history, clinical examination and lab investigations, the patient was diagnosed with HAE type I.

DISCUSSION

HAE is an autosomal dominant condition; ho wever, it can also arise from new mutations in the SERPING-1 gene [6]. 85% of individuals with HAE have a C1 INH deficit, 15% have dysfunctional C1 esterase inhibitor, and 6% have abnormalities in the regulators of the kinin system, which result in dysregulation of the complement and kallikrein-kinin systems [6]. HAE often manifests as recurring episodes of swelling across the face, limbs, digestive system, and airway that start in late childhood and typically get worse around puberty. Stress, exhaustion, infections, trauma, and medicines may cause these acute attack episodes, but swelling can also occur in the absence of the aforementioned established triggers [7]. An individual may experience acute attacks every week in the absence of treatment, with the majority of episodes lasting two to three days. HAE manifests similarly to other cutaneous conditions as urticaria, medication eruptions and acquired angioedema. In our case, the patient only displayed gastrointestinal symptoms and there were no laryngeal edema or cutaneous manifestations making it challenging to determine the underlying aetiology.

The gastrointestinal symptoms, which might i nclude nausea, diarrhea, vomiting, and/ or colic pain in varied degrees, are caused by edema of the colon wall. In contrast, during acute abdominal episodes, neutrophilia, hypovolemia from fluid losses, or hemoconcentration from plasma extravasation may mimic peritonitis. The absence of peritoneal symptoms, fever and neutrophilia may help to distinguish it from peritonitis.

TREATMENT

Avoiding triggers, taking short-term and long-term prophylaxis to lessen the frequency and severity of repeated attacks, and treating acute attacks are all possible strategies of managing HAE.

FFPs, anabolic androgens, and antifibrinolytics were t he three main therapeutic options available for HAE before 2009 [8]. But now Icatibant (bradykinin B2 receptor antagonist), Ecallantide (plasma kallikrein inhibitor) and C1-INH are considered first line treatment whereas solvent detergent treated (SDT) plasma and FFPs are second line treatment for acute attacks. Short term prophylaxis includes Plasma derived (Pd) C1-INH, FFPs and modified androgens. Long term prophylaxis incorporates Pd C1-INH, lanadelumab and Berotralstat. Gene therapy trials are also in progress [9].

Our patient was treated with fresh frozen plasma (FFPs) at a dose of 20 ml/kg as no medicine was available in Pakistan for acute attacks. Patient was under observation for 48 hours in hospital and was then discharged in a stable condition. He was also advised to take tablet Danazol 200 mg x OD in routine and FFPs in acute attacks and to avoid common triggers like stress, fatigue, infections, prolonged physical activities, minor physical trauma and ACE inhibitors.

CONCLUSION

The patient reported a noticeable improvement in his symptoms during follow-up visits over a six-month period, with a notable reduction in the frequency of episodes of abdominal pain and no further development of laryngeal oedema.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION

Mustajab Alam: Prepared and presented the case. Wrote the case report

Muhammad Hussain, Hamid Nawaz Tipu: Supervised the case report and did final reading and editing

Muhammad Aftab Hassan: Critical revision, literature search

Usama Bin Khalid and Hafiza Monaza Batool: Literature review

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GUIDLINES FOR AUTHORS

Pakistan Journal of Pathology (PAK J PATHOL) is the official journal of Pakistan association of Pathologists. Acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership.

1. Editorial Policy

The Journal will publish research material of interest to the researchers, scientists and medical practitioners. The Journal publishes peer-reviewed original papers, reviews, case reports, and editorials concerned with clinical practice and research in the fields of pathology. Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors'. In preparing manuscripts, authors should follow the "Uniform Requirements for Manuscript Submitted to Biomedical Journals updated October 2008, available at www.icmje.org" and specific author instructions detailed below.

2. Manuscript Submission

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(3) Please read checklist and make sure that no point in the checklist is missing

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(ii) Upload submission,

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