Original Article

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

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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.

This article can be cited as: Rasheed A, Khan MD, Batool H, Chughtai O, Chughtai AS, Ashraf S. Reference interval of placental growth factor in non-pregnant and pregnant females. Pak J Pathol. 2023; 34(4): 102-108.

DOI: 10.55629/pakjpathol.v34i4.772

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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Received: ***07 Jun 2023****; Revised:* ***10 Oct 2023****; Accepted:* ***07 Dec 2023***

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

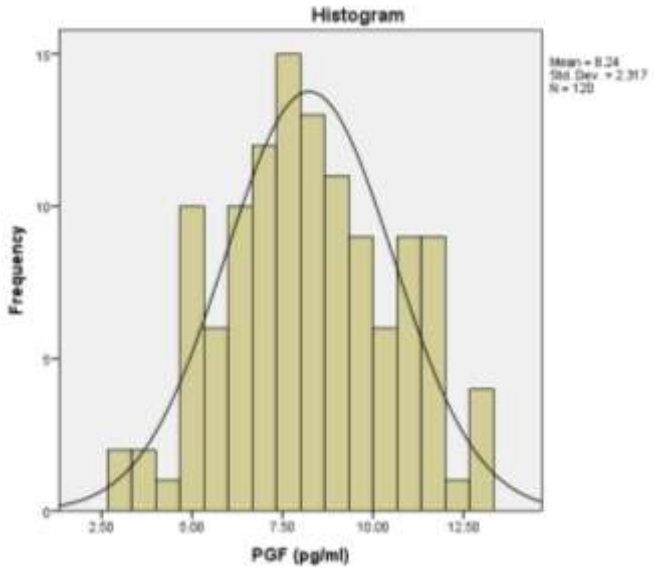

Figure-I: Histogram of PGF level (pg/mL) in healthy non-pregnant females

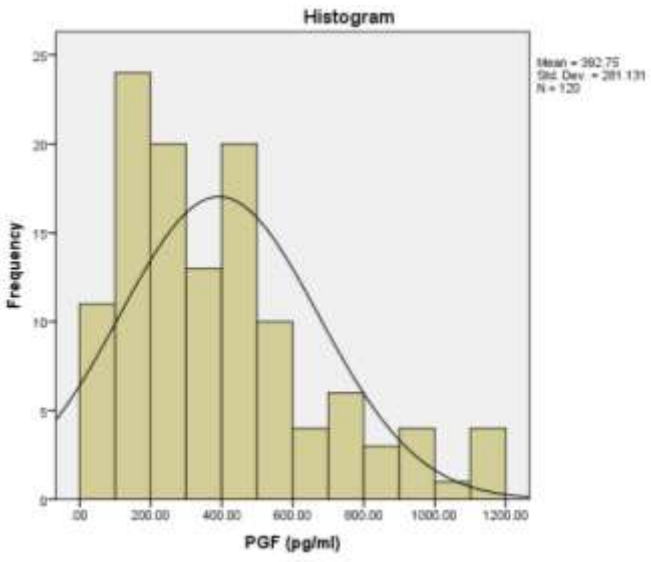

Figure-II: Histogram of PGF levels (pg/mL) in healthy pregnant females

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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