Original Research Article

A comparative study on ABO blood group and fertility hormones in infertile women in Calabar, Southern Nigeria

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Abstract

Infertility, a disorder of the reproductive system, is commonly linked to hormonal, pituitary, cervical, uterine, immunological or psychological factors. Besides these factors, it can also be idiopathic or unexplained. Hence, there is a need for more research to unravel the causes of the unexplained infertility. This work aimed at finding out whether there is any relationship between ABO blood group system and female infertility. The study design was cross-sectional. Three hundred women between 18 and 40 years attending fertility clinic at the University of Calabar Teaching Hospital, Calabar between 2011 and 2012 were recruited for this study. Serum progesterone, prolactin, follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol were determined using Enzyme linked immunosorbent assay (ELISA) while ABO blood grouping was determined using the tube method. Statistical analysis was done using SPSS version 18. The confidence level was set at 95% where p-value less than 0.05 was considered statistically significant. The mean age of the women was 30.65 ± 5.47 years and the percentage distributions of the blood groups among the women were as follows: 24% A, 12% B, 4% AB and 60% O. The mean FSH levels of blood groups A and O individuals were significantly higher (p<0.05) than that of groups B. However, there was no significant difference (p>0.05) in the mean levels of progesterone, prolactin, LH and estradiol in the respective groups. From this study, 38% of the population had increased levels of progesterone, 58% and 18.7% had elevated prolactin and FSH levels respectively while 11.33% and 43.3% had reduced levels of LH and estradiol levels respectively. Though, there was high prevalence of hyperprolactinemia observed in this study, there was no strong association between ABO blood group and female infertility but, the increased FSH levels observed in blood groups A and O may be a potential link between blood group and infertility and therefore may be beneficial for further study.

Introduction

Fertility can be referred to as childbearing performance while fecundity means childbearing ability although both terms are used interchangeably [1]. Ideally in most societies once a marital relationship is established between a man and a woman their expectation and that of their

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relatives as well is reproduction which follows in the sequence of conception, pregnancy and a healthy live birth. No deep thought is put into the physiology and processes of reproduction if maternity is hitch-free [2]. However, sometimes apparently healthy couples experience a condition of inability to conceive after 12 months or more of regular unprotected sexual intercourse. This condition is often referred to as infertility [3]. Infertility as a spectrum of reproductive failure is a social, economic, psychological as well as medical problem [4]. The incidence of infertility in a population has important demographic and health implications as high infertility has a dampening effect on population growth rate [3]. As a result of the social and psychological consequences of infertility, women have been known to manifest various psycho-sexual disorders while some may have decreased sexual drive and some may seek multiple sexual partners in an attempt to achieve a desired conception [5]. The World Health Organization (WHO) estimates that 8-12% of couples globally experience problems in conception [6]. Psychologically, infertility investigation probes into the most intimate and private life of an individual as the history involves questions about the patient's sex life, menstrual cycles of females and examination commonly incorporates genital examinations [7]. The common factors described to be the causes of female infertility include ovarian or hormonal factors, pituitary, cervical, uterine, immunological, psychological and iatrogenic factors [8]. In addition to the factors above, infertility can be idiopathic; in such cases it is loosely termed unexplained infertility [9]. That is why effort is demanded on research to unravel the latent cause of the unexplained infertility as a step in tailoring the solutions to patients looking for assistance [10]. There are a lot of contradicting reports on the relationship of ABO blood groups with female fertility. A study conducted in China suggested that women with blood types A and AB were at increased risk of Diminished Ovarian Reserve (DOR) while those with type O were at low risk. Although, this is in contrast to the findings of Nejat et al who conducted a similar study in the United States of America (USA) and suggested that A blood group antigen was protective against DOR in sub-fertile women than the O blood group antigens [11]. While another study by Timberlake et al in the USA and Yilmaz et al in Turkey reported that they found no clinical implications for particular blood type as a risk factor for DOR [12,13]. However, the underlying mechanisms behind these findings are still not clear. These studies were on Caucasian and Asian populations. Therefore, this work investigated the relationship if any between ABO blood group and infertility in African women.

Materials and methods

Study design and subject selection

The design of the study was cross-sectional. A total of three hundred women participated in this study. The participants were consecutively recruited from women of reproductive age (18-40 years) attending the fertility clinic at University of Calabar Teaching Hospital (UCTH), Calabar in Southern Nigeria. All the subjects were infertile and any subject above forty years of age was excluded. Ethical approval was obtained from the Ethics committee of the University of Calabar Teaching Hospital, Calabar (RP/REC/2015/311). The purpose and nature of the research was explained to the participants and informed consent was obtained from them.

Sample size calculation

The number of samples used in this research was determined using the Leslie Kish formula [14]

$$N = \frac{Z\alpha^2 pq}{d^2}$$

where N = desired sample size
Zα = the α level of the coefficient interval at 95% (1.96)
p = proportion of occurrence
q = (1-p) proportion of non-occurrence
d = precision

Substituting the expected occurrence of p=12% i.e. 0.12 from WHO [6], we have

$$N = \frac{(1.96)^2 \times 0.12(1 - 0.12)}{(0.05)^2} = 162.3$$

Sample collection

On the 21st day of their menstrual cycle, four milliliters (4 ml) of blood were aseptically collected from each subject into a plain container and allowed to clot at room temperature. The samples were spun at 3000 rpm for 10 minutes. The supernatant sera were then harvested into clean specimen containers and labeled accordingly. The sera were stored frozen at 4ºC until needed for assay of the hormonal parameters. Some of the serum and cells were used immediately for serum and cell grouping respectively.

Estimation of hormones and ABO blood group determination

Serum progesterone, prolactin, follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol were analyzed using Enzyme Linked Immunosorbent Assay (ELISA) kits obtained from Syntron Bioresearch Incorporated Carlsbad, California. The analyses were carried out using the

manufacturer's instructions. ABO cell grouping were analyzed with antisera produced by Carper Laboratories and Down Patrick Technology both in United Kingdom while serum grouping was done using the four tube method and standard cells. Both were carried out according to standard procedure.

Statistical analysis

This was done using the PAWS 18, a statistical package from SPSS Inc, Chicago, USA. The results were expressed as Mean ± SEM. The data was analyzed using one way analysis of variance (ANOVA) and the significance level was set at 95% confidence interval where p-value less than 0.05 ($p < 0.05$) was considered as statistically significant.

Results

Table 1 shows the distribution of ABO blood group. Among the 300 female subjects used for this study, 73(24%) had blood group A, 36(12%) had blood group B, 11(4%) had blood group AB while 180 (60%) had blood group O. There was no significant differences ($p < 0.05$) in the mean age of the subjects in the respective blood groups. A comparison of the mean serum values of the hormones; progesterone, prolactin, luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol of the blood groups A, B, AB and O showed that the mean FSH levels of subjects with blood groups O and A were significantly higher ($p < 0.05$) than those with blood group B (**Table 1** and **Table 2**) while there was no significant difference ($p > 0.05$) in the mean serum levels of progesterone, prolactin, LH and estradiol of the different blood groups. **Table 3** shows the percentage distribution of the different hormones of the subjects with different blood groups within normal, above normal and below normal reference limits. More than 22% of blood group O subjects had elevated FSH levels while 50% had reduced estradiol levels. Hyperprolactinemia was observed across all the groups with group B having the highest distribution (69%) and group A with the lowest (55%). A reduction in the LH levels was also observed in more than 45% of each group. **Table 4** shows the percentage distribution of hormones in the sample population. Thirty eight percent (38%) of the total population had increased progesterone levels though more than 50% had normal progesterone levels. Fifty eight percent (58%) had elevated prolactin levels in the study. Low levels of estradiol were observed in 43.3% of the study population while 18.7% and 53.3% had increased levels of FSH and LH respectively.

Table 1: Comparison of mean values of the hormones and blood groups A, B, AB and O of the infertile women

Blood group	n (%)	df	Age (years)	Progesterone (ng/ml)	Prolactin (ng/ml)	LH (ng/ml)	FSH (ng/ml)	Estradiol (pg/ml)
A	73 (24)	3.296	31.62±0.63	8.96±1.19	36.73±4.47	9.71±1.52	18.14±3.29*	76.64±5.43
B	36 (12)	3.296	30.18±0.86	10.24±1.61	37.81±4.39	5.41±0.76	6.54±2.3	80.94±10.55
AB	11 (4)	3.296	29.18±1.87	10.55±3.08	28.08±5.30	7.67±3.01	6.73±2.19	80.55±13.49
O	180 (60)	3.296	30.39±0.41	9.70±0.93	41.20±4.29	9.22±0.86	17.61±2.11**	73.83±3.78
f-cal			1.177	0.140	0.356	1.360	2.139	0.250
f-critical			2.65	2.65	2.65	2.65	2.65	2.65
p value			>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Remarks			NS	NS	NS	NS	NS	NS

Numbers represent X ± SEM where X=mean, SEM = standard error of mean
NS = Not significant, df = degree of freedom, p = probability level
*FSH level of Blood group A is significantly higher than that of B
**FSH level of Blood group O is significantly higher than that of B

Table 2: Comparison of mean FSH (ng/ml) levels in different blood groups using Post hoc analysis

Blood groups	Mean difference	Std. error	p
A vs B	11.592	5.673	0.042
A vs AB	11.395	9.008	0.207
O vs AB	10.950	8.652	0.207
B vs O	11.147	5.088	0.029

Table 3: Percentage distribution of hormones (progesterone, prolactin, LH, FSH and estradiol) of subjects with different blood groups within normal, below and above the reference limits

Blood group	Hormone (reference limit)	n	Normal (%)	Below (%)	Elevated (%)
O	Progesterone (2.5-2ng/ml)	180	105 (56)	70 (38)	5(3)
	Prolactin (8.39-20.15ng/ml)	180	60 (33)	17(10)	103(57)
	LH (5-20ng/ml)	180	58 (32.2)	99 (55)	34 (12.8)
	FSH (<20ng/ml)	180	139 (77.2)	0(0)	41 (22.8)
	Estradiol (60-150pg/ml)	180	76(46.7)	90 (50)	14 (7.8)
A	Progesterone (2.5-32ng/ml)	73	42 (58)	27 (37)	4(5)
	Prolactin (8.39-20.15ng/ml)	73	26 (36)	7 (9)	40 (55)
	LH (5-20ng/ml)	73	31(42.5)	33 (45.2)	9(12.3)
	FSH (<20ng/ml)	73	60(82.2)	0 (0)	13(17.8)
	Estradiol (60-150pg/ml)	73	41 (56.2)	27 (37)	5(6.8)
B	Progesterone (2.5-32ng/ml)	36	23 (64)	12 (33)	1 (3)
	Prolactin (8.39-20.15ng/ml)	36	9 (25)	2 (6)	25 (69)
	LH (5-20ng/ml)	36	12 (33.3)	23(63.9)	1 (2.8)
	FSH (<20ng/ml)	36	35 (97.2)	0(0)	1 (2.8)
	Estradiol (60-150pg/ml)	36	18 (50)	14 (38.9)	5 (6.8)
AB	Progesterone (2.5-32ng/ml)	11	6 (55)	4 (36)	1 (9)
	Prolactin (8.39-20.15ng/ml)	11	4 (36)	0 (0)	7 (64)
	LH (5-20ng/ml)	11	5 (45.5)	5 (45.5)	1 (9)
	FSH (<20ng/ml)	11	10 (90.9)	0(0)	1 (9.1)
	Estradiol (60-150pg/ml)	11	5 (45.5)	5(45.5)	1 (9)

Table 4: The percentage distribution of hormones (progesterone, prolactin, LH, FSH and estradiol) in the sample population within normal, above, and below the reference limits

Hormone (reference limits)	Normal (%)	Above (%)	Below (%)	Total (100%)
Progesterone (2.5-32ng/ml)	176 (58)	113 (38)	11 (4)	300 (100)
Prolactin (8.39-20.15ng/ml)	99 (33)	175 (58)	26 (9)	300 (100)
LH (5-20 ng/ml)	106 (35.3)	160 (53.3)	34(11.33)	300 (100)
FSH (<20 ng/ml)	244 (81.3)	56 (18.7)	0(0)	300 (100)
Estradiol (60-150pg/ml)	140 (46.7)	24 (8)	136 (43.3)	300 (100)

Discussion

Female fertility is controlled by the hypothalamus-pituitary-ovarian-axis [15]. In this research work, the luteal phase (21st day of menstrual cycle) levels of the gonadotrophic pituitary and ovarian hormones were measured in three hundred (300) female subjects to investigate a relationship between ABO blood group system and female fertility. The distribution of ABO blood group among them showed that 73(24%) had blood group A, 36(12%) had blood group B, 11(4%) had blood group AB while 180 (60%) had blood group O.

From the study, up to 85.3 % of the subjects had one or two or more hormonal abnormalities while 14.7% had no observable hormonal abnormalities. This agrees with the findings of Korhonen et al who reported over 90% hormonal abnormality in women and are under hormone supplementation therapy [16]. Also, there was no significant variation ($p<0.05$) in the mean serum levels of the hormones (progesterone, prolactin, LH, FSH and estradiol) among the different groups. However, the mean FSH levels of blood groups O and A were significantly higher than that of blood groups

B and AB. This agrees partly with the findings of Nejat et al who found out that those with blood group O were more likely to have higher levels of FSH than those of other blood groups. Fertility experts regard a high FSH level as a key indicator of having a low ova count, which is known as Diminished Ovarian Reserve (DOR). This puts them at risk of having fertility problems later in life [11]. One hypothesis to explain this finding is that the same molecular machinery that determines blood group type could also effect ovarian cells. The red blood cells of subjects with blood groups A, B and AB have enzymes that modify their cell surface antigens which are absent in blood group O subjects. It is believed that these enzymes may also have some unknown functions in ovarian reserve and infertility though there is a little hard evidence to support this [11]. As one of the fertility markers, continuous elevation of the basal FSH levels reveals a reduced feedback inhibition of the estradiol and inhibin B of the ovary [17]. This may be why 50% of O group have subnormal level of estradiol. These are indications of poor ovarian reserve or premature ovarian failure or premature menopause [18]. This finding is in contrast to a recent Chinese study by Shapiro and Bressler which reported that blood types A and AB were associated with a greater likelihood of DOR while blood type O was associated with lesser likelihood [19]. Although the exact mechanisms that tie blood type to ovarian reserve and infertility are unclear, the possibilities that glycosyltransferases may play an important role in ovarian function or that the presence of a gene that co-segregates with DOR, are both enticing and constitute an area for further investigations [11]. In summary, from this study, 38% of the sample population had reduced levels of progesterone, suggestive of anovulation and amenorrhea. 58% had elevated prolactin level while 53.3% had reduced levels of LH which is seen in the cases of hyperprolactinemia and 45.3% had depressed estradiol levels.

Conclusion

Though, a high prevalence of hyperprolactinemia among the infertile women was observed, a strong evidence of relationship between female infertility and ABO blood group system was not established from this study but, the increased FSH levels observed in blood groups A and O may be a potential link between blood group and infertility and therefore may require further study.

Limitation of the study

In our study, day 21 of the participants' menstrual cycle was considered as all of them came to the clinic for luteal function assessment. Those for ovarian reserve function that came to the clinic on day 2 or 3 were few in number and were excluded from the study in order to maintain uniformity and obtain a larger sample size. We recommend that further studies be carried out, with hormonal profile on day 2 or day 3 of the participants' menstrual cycle taken into consideration.

Competing interest

The authors declare that they have no competing interests.

Ethical approval

All authors hereby declare that the study have been approved by the University of Calabar Teaching Hospital Ethics Research Committee (RP/REC/2015/311) and therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Authors' contributions

This work was carried out in collaboration between all authors. Authors IEB and NNN initiated and designed the research. Authors NNN and SJO did the analysis and interpretation of data. Authors BIE and UOA wrote initial draft manuscript while authors SJO, IEB and UOA critically reviewed the manuscript. All authors read and approved the final manuscript.

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