



Study of distribution of allelic and phenotypic frequencies of ABO and Rhesus Rh in Côte d'Ivoire

F. S. Ouattara-Soro¹, F. N'Gou¹, K. L. Siransy-Bogui² and B. Dembélé²

¹Laboratory of Endocrinology and Reproduction Biology /University Felix Houphouët Boigny, 22 Bp 582 Abidjan 22 /Abidjan-Côte d'Ivoire

²National Blood Transfusion Center of Côte d'Ivoire/ University Felix Houphouët Boigny 22 Bp 582 Abidjan 22/ Abidjan-Cote d'Ivoire

ABSTRACT

Since the discovery of the first ABO blood group, over 33 blood group systems have been identified. The study of this system for transfusion requirements demonstrates the existence of the variations among human populations. To better meet these needs, this study looked at the distribution of phenotypic and allelic frequencies of ABO and Rh Rhesus in Côte d'Ivoire. Thus, the results show a frequency of 50,97% of the phenotype O. This phenotype is dominant in all districts from Côte d'Ivoire. It is followed by the B phenotype at a frequency of 23,18%, then the phenotype A to 21,73%, and finally the AB phenotype to 4,12%. Also the frequencies of phenotypes A and B are substantially equal in almost all districts except in the districts of the savannah and Bandaman Valley. All phenotypic frequencies are much higher than those phenotypes of Group AB. Statistical analysis of variance revealed significant differences between the phenotype A, B, AB and O ($\chi^2_{cal} = 38,143$; DDL = 3, $p < 0,0001$). This study thus indicates that the genotypic polymorphism ABO and RH (D) in the Ivorian population is similar to that of the black populations of Africa and America. Also, it allows us to establish a gradient distribution of ABO blood group and Rh by district. This is to raise awareness of the Ivorian people donated blood.

Keywords: Côte d'Ivoire, distribution, ABO blood group and Rh

INTRODUCTION

The history of blood transfusion started since the 15th century. In 1667, Jean Baptiste DENIS, medical personal to Louis XIV practice blood transfusion for the first time with the blood of a lamb [1]. The term blood had already been the subject of study and the principle was also described by William HARVEY thanks to the discovery of Circulatory Physiology. The first human blood transfusion was done in 1818 followed by considerable consequences ie incompatibilities, transfused blood clotting and transmission of pathogens. The work of Karl Landsteiner in 1900 revealed the existence of the ABO system [2], the blood transfusion of a man to another becomes possible. This work marks the beginning of erythrocyte immunology with the creation of the first blood bank in 1940 in France [3]. They also mark the beginning of the expansion of blood centers in the world. Furthermore, blood groups correspond to the erythrocyte membrane antigens and are the expression of the detectable human genetic variability in the blood [4]. They have a fundamental interest in many scientific and pathological areas, including medical immunology, medical pathology, human genetics and blood typing [5; 6]. Since the discovery of the first ABO blood group in 1900 by Karl Landsteiner, over 33 blood group systems have been identified [7]. The study of this system for early transfusion requirements demonstrates the existence of the variations among human populations [8; 9; 10]. The distribution of the alleles in the world system has been extensively studied [11; 12; 13]. It is often associated with the development of genetic structure of human populations and also to natural selection [3; 14; 9]. In 1982, Vogel and Motulski have linked the global distribution of ABO polymorphism to major epidemics and infectious diseases [15]. The reasons of frequency variations in of the ABO gene from one population to another are not well

known [13; 16]. However, most of these blood factors are not indifferent to the living environment and exert a selective value [17]. In Asians, a relatively high frequency of allele B is the result of a double selective action of two diseases; the plague for the O allele and smallpox for the A allele [18; 10]. Thus, according to some studies, blood type O are favorable to disease caused by bacterial strains of *Escherichia coli* such as cholera and infant diarrhea [19; 20]. As tuberculosis, gastric and duodenal ulcer are frequently found in people of group A [21; 22; 23]. Moreover, in Côte d'Ivoire, studies including the distribution of blood groups were performed [24] and they indicate that the AB phenotype was rare in this country. The aim of our study is to map blood group ABO and Rh systems in Côte d'Ivoire. To do so, we will determine the phenotypic frequencies of ABO and Rh and allele frequencies of these systems in Côte d'Ivoire.

EXPERIMENTAL SECTION

This study was conducted from June 2013 to June 2014 at the National Blood Transfusion Center (CNTS) in Abidjan. It aims to map blood group ABO and Rh systems in Côte d'Ivoire.

*Study circles

The laboratory of the National Blood Transfusion Centre of Abidjan generates about 84% of qualification tests of donations collected in Côte d'Ivoire [24]. Attendance at this center for blood donors has increased dramatically in recent years with an average of 98.000 donations per year [25]. The CNTS general activity is summarized in two main objectives namely the production and distribution of blood products. In addition, the laboratory of the National Blood Transfusion Centre of Abidjan also ensures the quality and reliability of blood products available to the population. This experimental study was conducted over a period of 12 months, from June 2013 to June 2014. This is a retrospective study on the description of phenotypic and genotypic frequencies of 39.019 blood donors in Abidjan's CNTS and in the fixed collection centers of blood transfusions in Côte d'Ivoire. Regarding the inclusion criteria for this study, the donor should be aged between 18 and 60, to fast and have a weight of over 45 Kg. Women in pregnancy and breastfeeding women were excluded.

*Technical equipment

The technical equipment used includes the collection hardware, metering devices and reagents. In a conditioned room, blood samples were taken by venipuncture using needles. The blood sample is collected in hemolysis tubes of 5 ml containing EDTA as anticoagulant.

Racks are used to classify blood samples based on the bar code assigned to the donor. Microplates and grouping plates used as support for determining blood groups were agitated with the agitator Kline IQM. The centrifuge GRIFOLIS gel card is used to centrifuge the blood samples. A centrifuge BR4i Multifunction was used to separate plasma from blood elements. The incubator GRIFOLIS gel card is used for the various incubations. A computer connected to a bar code reader captures the results after the blood typing of donors. In addition, the reagents used were provided by the laboratory DIAGAST. These reagents are among others, the test red blood cells A and B, serum tests namely the anti-A, anti-B, anti-AB and anti-D antisera. These reagents are also versatile anti globulin, neutral gel card and 0,9% physiological saline.

*Methodology

For the ABO-Rh typing, blood samples were centrifuged at 3000 rpm / min during 3 min to obtain plasma. Grouping the plate is then placed vertically on the pallet, and one drop of each antiserum A, anti B, and anti AB anti D is horizontally placed in the first four wells of the plate in order to highlight the system antigens OBA the surface of red blood cells by the globular technique Beth Vincent. On the same plate, two drops of the same plasma sample to be tested are filed in the last two wells for the highlight of ABO antibodies content in the plasma from the plasma technique Simonin-Michon. The grouping of plate thus prepared is stirred with the stirrer to Kline homogenization for 3min. Reading the results are at the end of 3 minutes. In the presence of agglutination, the result is positive and in the absence of agglutination, the result is negative. On research the low D or "D^{low}", the red blood cells to be tested undergo three washes with saline. These red blood cells were suspended by adding 400 µl of physiological saline at 50 µl of original blood. Two anti-D serum drops are added to each well of the gel card. 50 µl of the erythrocyte mixture and saline are then removed and placed in a well of the gel card containing the antiserum D. The mixture is then incubated at 37 ° C for 15 min. At the end of the incubation, a drop of anti-globulin is placed in each well and the resulting mixture is centrifuged for 10 minutes at 990 revolutions / min. The reading of the results is done by observation. In the presence of a ring at the surface of the gel, the result is positive when the result is negative, a deposit is observed at the well bottom. Determination of phenotypic frequencies. Phenotypic frequencies of ABO and Rh were calculated using the following formula:

F = Number of individuals possessing a specific antigenic character / Number Total

*Determination of allelic frequencies of the ABO system

Gene frequencies of alleles A, B and O have been calculated by estimating the efficient calculation method of Bernstein and Steven.

- Step 1: preliminary estimate calculation

$$p' = 1 - \sqrt{O + B}$$

$$q' = 1 - \sqrt{O + A}$$

$$r' = \sqrt{O}$$

With O, A and B respectively designate the frequencies of phenotypes O, A and B.

- Step 2: Calculate the constant D

$$D = 1 - (p' + q' + r')$$

- Step 3: Frequency Adjustment. The adjusted frequencies are obtained by the following formulas

$$p = p' \left(1 + \frac{D}{2}\right)$$

$$q = q' \left(1 + \frac{D}{2}\right)$$

$$r = \left(r' + \frac{D}{2}\right) \left(1 + \frac{D}{2}\right)$$

*Determination of allelic frequencies of Rh system.

Gene allele frequencies RhD and Rhd were calculated by the method of Landsteiner Wiener and [26].

$$d = \sqrt{Rh}$$

$$D = 1 - \sqrt{Rh}$$

with:

d = allele frequencies corresponding to the negative Rh;

D = allele frequencies corresponding to the positive Rh;

Rh = Phenotypic frequencies corresponding to the negative Rh

*Statistical analysis of the data

The data statistical analysis was carried out with the XLSTAT software with the use of different tests namely the z-test for comparison of two proportions, the k of test comparison of proportions and the independence of Chi2 test at the significance threshold 5%.

RESULTS

The comparison of phenotypic frequencies diagram A, B, AB and O Figure 1 shows a frequency of 50,97% of the phenotype O. This phenotype is dominant in all districts. It is followed by the B phenotype at a frequency of 23,18%, then the phenotype A to 21,73%, and finally the phenotype AB to 4,12%. Also the frequencies of phenotypes A and B are substantially equal in almost all districts except in the districts of the savannah and Bandaman Valley. All phenotypic frequencies are much higher than those phenotypes Group AB (Fig. 1). Statistical analysis of variance revealed significant differences between the phenotypic frequencies A, B, AB and O ($\chi^2_{cal} = 38,14$; DDL = 3, $p < 0,0001$). Moreover, the two-proportion z test indicates that there is no significant difference between the frequencies of phenotypic groups A and B except in the districts of the savannah with $p = 0,001$ and the Bandaman Valley where $p < 0,0001$. The study of the phenotype A répartition in Côte d'Ivoires shows that the highest frequencies are found in the districts of Woroba 29,41% and Bas-Sassandra to 24,25%. As against the lower frequencies are recorded in the two districts of Zanzan to 20,31% and Yamoussoukro to 20,31% (Fig. 1). Statistical tests showed this phenotype for significant differences between some districts namely districts Woroba and lagoons with ($p = 0,01 < 0,05$), and the Woroba Yamoussoukro where ($p = 0,004 < 0,05$), Bas Sassandra and Valley Bandaman with ($p = 0,009 < 0,05$) finally Bas Sassandra and Abidjan ($p = 0,049 < 0,05$). The analysis of the distribution of blood groups B phenotypes indicates significant differences in phenotypic frequencies between several districts. The highest phenotypic frequencies are recorded in the districts of North, West and Central districts namely Denguelé to 27,33%; the Woroba to 28,88% and 30,44% in the Savannah (Fig. 1). As against the lower phenotypic frequencies are recorded in the districts Lagoons to 20,7%; Abidjan to 22,01%, with 19,57% of the Comoé and Zanzan to 21,44% (Fig. 1). The statistical analysis for this phenotype shows that there are significant differences between some districts with ($p < 0,001$). Thus, between the districts of Savannah and lagoons, it was

noted ($p = 0,001 < 0,05$), Yamoussoukro and Abidjan one difference of ($p = 0,039 < 0,05$) finally Zanzan and Woroba ($p = 0,026 < 0,05$). Regarding the frequency of the phenotype AB, the study indicates a high frequency to 7,45% in Denguélé district. Furthermore, the phenotype frequencies of the lowest group were observed in the districts of Woroba to 2,14%; Mountains of 3,03% and 3,9% of Abidjan (Fig. 1). The statistical results of the phenotype AB therefore presents significant differences between the Denguélé district and that of Woroba with ($p = 0,018 < 0,05$). Also for this phenotype, a difference was noted between the district of Abidjan and the Denguélé where ($p = 0,021 < 0,05$). The study of the distribution of O phenotypes indicates differences between several districts in phenotypic frequencies. These frequencies are higher in the southern districts namely District Lagoons at 53,35; in Abidjan to 52,4% and 54,24% in Comoé. For this group, the lowest phenotypic frequencies were observed in the districts of North and West among other District Savannah to 43,71% and the Woroba to 39,57% (Fig. 1). Statistical tests for the O phenotype show that there are significant differences between some districts. These differences are between the Denguélé district and those of Lagoons with ($p = 0,01 < 0,05$) and between the districts of Zanzan and Mountains where ($p = 0,006 < 0,05$).

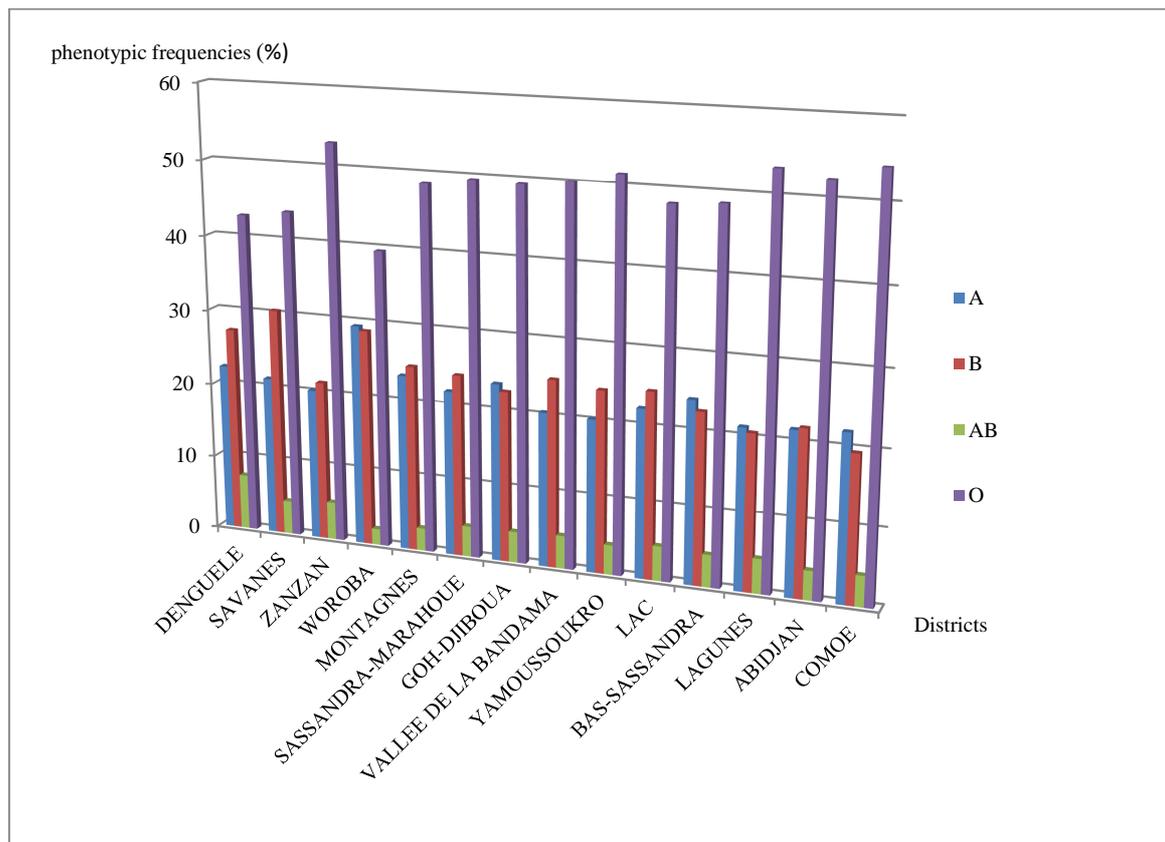


Fig. 1: Comparison of phenotypic frequencies diagram A, B, AB and O

The comparison of phenotypic frequencies diagram Rh (D) and Rh (d) of Figure 2 shows a significant gap between the phenotype Rh (D) positive or Rh at an average frequency of 92,95% and Rh phenotype (d) or Rh negative to the average frequency of 7,05%. The highest frequency of these phenotypes is found in the district of the mountains and the lowest in that of Abidjan. For this phenotype, statistical tests showed significant differences ($p < 0,001$) between the district of the Mountains and those of Abidjan.

The comparison of allelic frequency diagram AB and O in Figure 3 indicates a predominance of the O allele with a frequency of 0,72%. Regarding the alleles A and B with respective frequencies for 0,15% and 0,14%, they are almost identical in most districts except in Denguélé, Savannah and Bandama Valley districts.

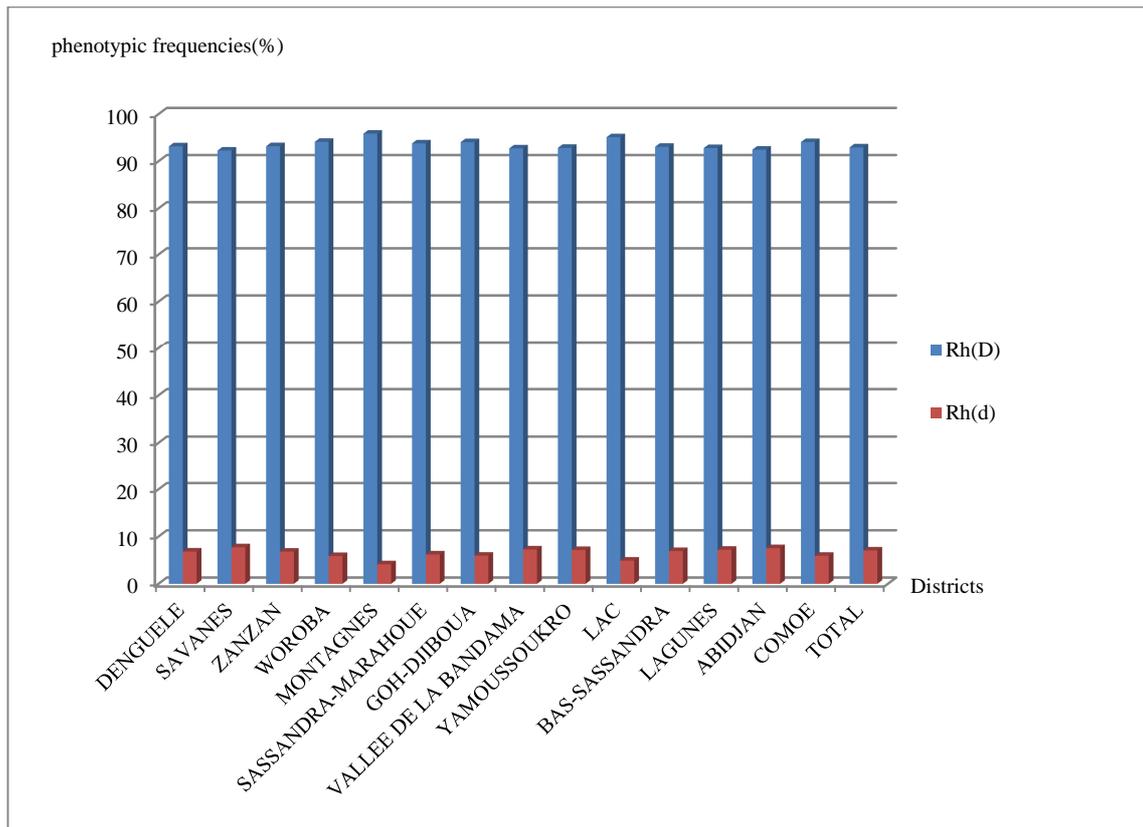


Fig. 2: Comparison of phenotypic frequencies diagram Rh (D), and Rh (d)

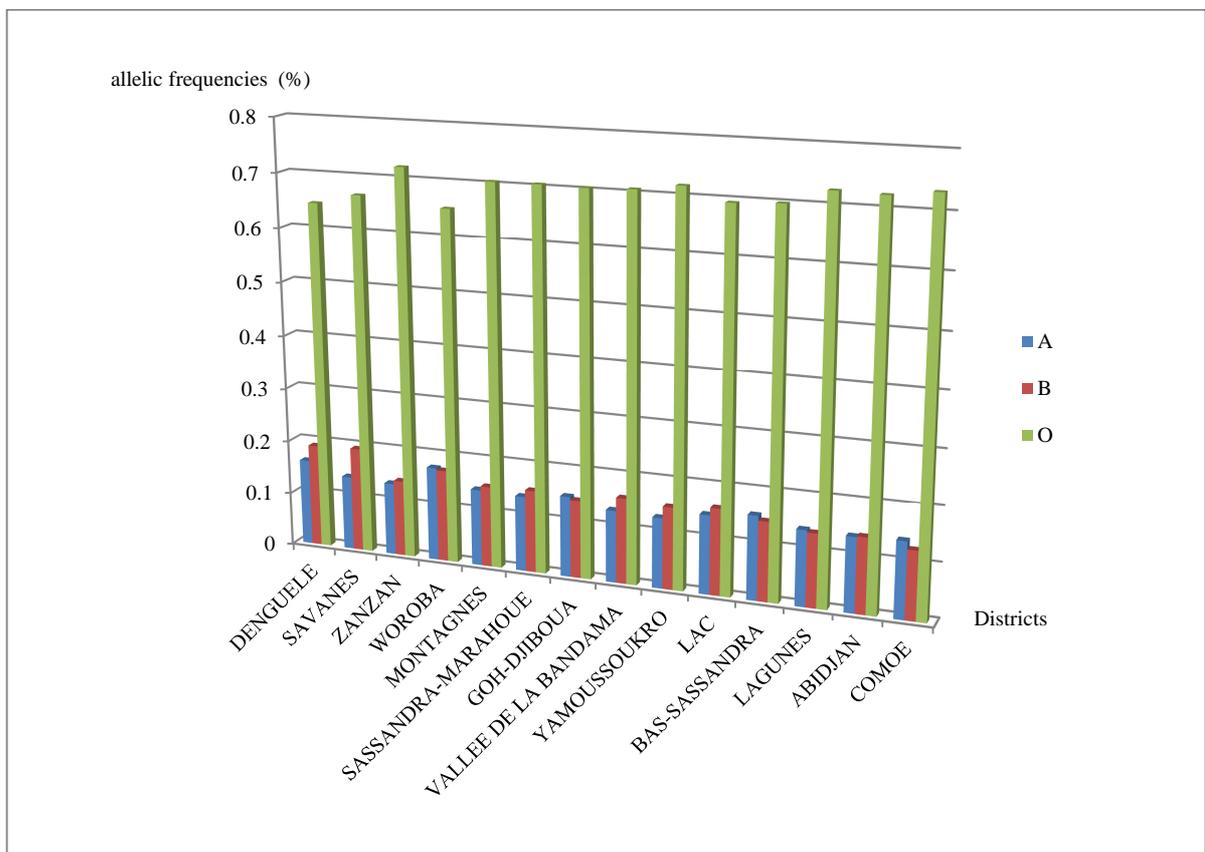


Fig. 3: Comparison of allelic frequencies diagram AB and O

The comparison of the allelic frequencies diagram Rh (D), and Rh (d) of Figure 4 shows the predominance of the D allele with a frequency of 0,73%

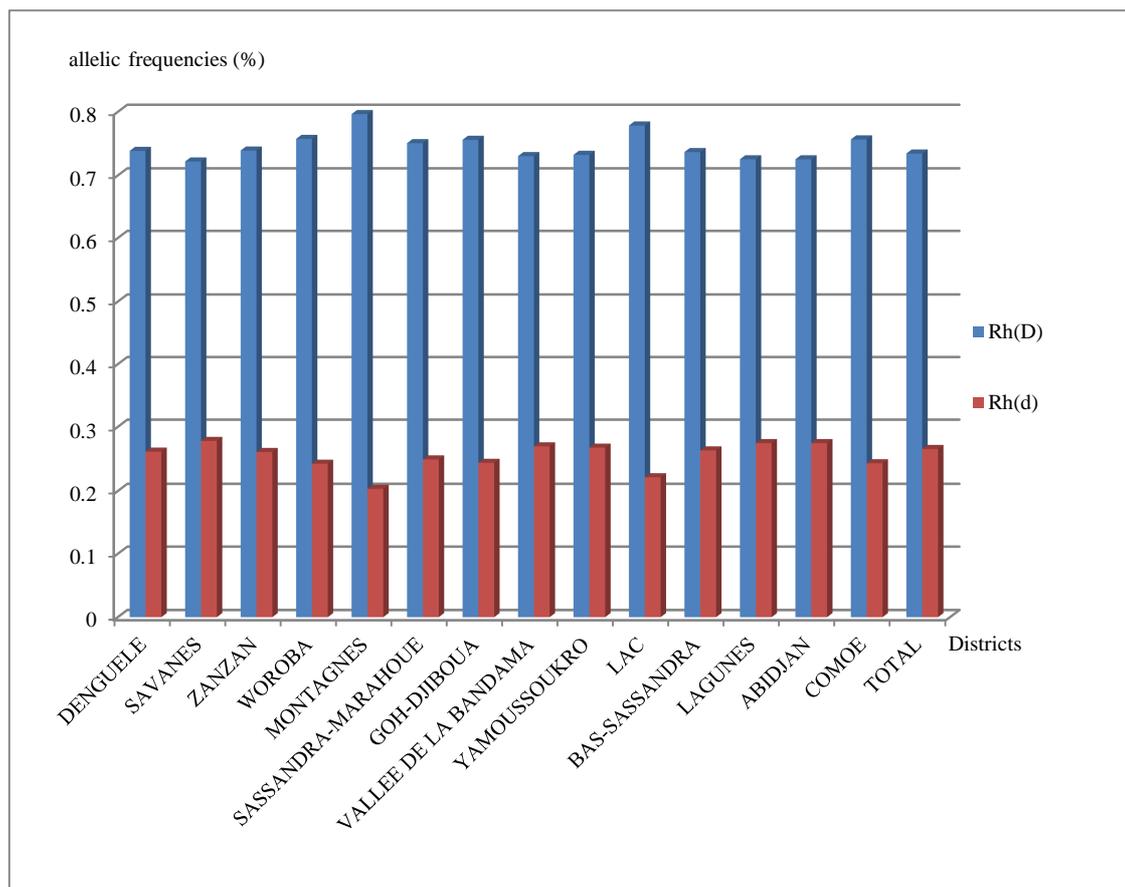


Figure 4: Comparison of the frequency diagram allelic Rh (D), and Rh (d)

DISCUSSION

The study of phenotypic frequencies of blood groups in the ABO system shows a predominance of group O in the Ivorian population with 50,97% followed group B, A and AB which have respective phenotypic frequencies of 23,18%; 21,73% and 4,12%. These results corroborate those of SOMBO [27] and those of Dulat [28] obtained at the Ivorian population. Also some of the results in black Africa [29; 30; 31] and America [32] show similar phenotypic frequency distribution. Furthermore, studies conducted in North Africa in Morocco [33; 34] indicate phenotypic frequencies in Group A higher more than 30% compared to those obtained in the Ivory Coast which is 23%. In the Caucasian population, the phenotype A is predominant with 43% of the ABO system [26]. Our results indicate an increasing gradient distribution of the frequency of the B antigen from south to north and from east to west. As for that of the O antigen, it decreases from south to north and from east to west. The frequency of the AB phenotype is higher in the Denguélé district. These results are similar to those of Dulat and Trolet in 1989 [28] but differ from those of Dembele and collaborators in 2009 [24] which have conducted their study in only five cities in Côte d'Ivoire. This difference could be explained by the number of city that was limited to 5. In terms of allele frequencies, the O allele is most encountered in the Ivorian population at a frequency of 71,38%. Alleles A and B allele frequencies have some similar. The gradient distribution of allele B increases from south to north and from east to west, while that of the O allele decreases in the same direction. The change in allele frequencies of a one district to another could be explained by the migration of populations [4]. In addition to it there's migration phenomena mutation and natural selection that could explain the genetic variation between populations [6; 35]. The result of allele frequencies of this study is similar to the results of some work reported in other sub-Saharan African populations [30; 36; 37; 34] and in the black population of America [34; 38]. In the populations of North Africa and the Caucasian populations of Europe and America, although the O genotype is predominant, the frequency of genotype A is higher than 20% that of genotype B [34; 38; 39; 40]. Côte d'Ivoire frequencies are similar to these around 15%. The positive rhesus D phenotype is dominant in the Ivorian population with a frequency of 92,95%. These results are similar to those of Sombo [27] as well as those of Dulat and Tolet [28; 41] who reported

frequencies of 94% of Rhesus D positive in the Ivorian population. This result also supports the work of Loua and Mwanji conducted in some black African countries [30; 31]. The frequency of the D allele in the Ivorian population to 73,45% is similar to that reported in Morocco 70% Sbiti and colleagues in 2002 [26]. Furthermore, the results reported by Falusi and collaborators in 2000 in a population of Nigerian blood donors have indicated a frequency to 81,50% of the D allele [42; 43]. These results confirm a large distribution of Rhesus positive phenotype in black African populations.

CONCLUSION

The study of the distribution of phenotypic frequencies A, B, AB and O revealed a frequency of 50,97% of the phenotype O. This phenotype is dominant in all districts in Côte d'Ivoire. It is followed by the B phenotype at a frequency of 23,18%, then the phenotype A to 21,73%, and finally the phenotype AB to 4,12%. Also the frequencies of phenotypes A and B are substantially equal in almost all districts except in the districts of the savannah and Bandaman Valley. All phenotypic frequencies are much higher than those of phenotypes Group AB. Statistical analysis of variance revealed significant differences between the phenotype A, B, AB and O ($\chi^2_{cal} = 38,143$; DDL = 3, $p < 0,0001$). Moreover, the two-proportion z test indicates that there is no significant difference between the frequencies of phenotypic groups A and B except in the district of Savannah with $p = 0,001$ and that of the Bandaman Valley where $p < 0,0001$. Thus, these results indicate that the genotypic polymorphism ABO and RH (D) in the Ivorian population is similar to that of the black populations of Africa and America. Also, this study allows us to establish a distribution gradient of blood groups ABO and Rh by district. This is to raise awareness of the Ivorian people donated blood.

REFERENCES

- [1] PCassus, *Une histoire de la transfusion Médecine* ; (2009), 5 (2), 87-90
- [2] J Lefrere, P Berche, *Transfusion, clinique et biologique* ; (2009), 17 (1): 18-35
- [3] K Landsteiner, P Levine, *Proc. Soc. Exp. Biol.*; (1927), 24:941-952.
- [4] A Fernandez Santander, M Kandil, F Luna, E Esteban, F Gimenez, D Zaoui and P Moral; *American Journal of biology*; (1999), 11: 745-752.
- [5] C Salomon, JP Carton, P Rouger, *Paris : Editions Masson* ; (1991), 537p
- [6] M Goudman, C Salmon, *Paris: Flammarion Médecine-Sciences*; (1980), 573 p.
- [7] J Chiaroni, V Ferrera, I Dettori, F Roubinet, *EMC-Hématologie*, (2005), 2 : 53-112.
- [8] M Lewis, DJ Anstee, GWG Bird, E Brodheim, JP Cartron, M Contreras, MC Crookston, W Dahr, G Daniels, CP Engelfriet, *Vox Sang.*; (1990), 58(2):152-169.
- [9] G Daniels, *Blackwell Science*; (2002), 2 ed. Oxford
- [10] JR Storry, ML Olsson, *Immunohematology*; (2009), 25(2):48-59.
- [11] J Reviron et M Reviron, *Encyclo. Med. Chir. (Paris, France)* ; (1984), 11 : p8.
- [12] ML Olsson, *Lund University*; (1997), 7-42.
- [13] NM Irshaid, *Lund University*; (2001), 8-20.
- [14] J Ruffie, JC Sournia, Ed. Fayard, coll. *Le temps des sciences*, (1996), p211
- [15] F Vogel and A Motulski, *Springer Verlag. Berlin*; (1982), p345
- [16] JP Cartron, *49ème journées de Biologie clinique* ; (2006), p25
- [17] J Ruffie, *Novelle encyclopaedia Universalis. 4ème Ed. V.* (1998), 11
- [18] F Yamamoto, *Immuno hematology*; (2004), 20 (1):13-22.
- [19] A Najman, E Verdy, G Paton, P Isnard, *Précis des maladies du sang.* (1994), T1 et T2. Ellipes.
- [20] A Sanchez-mazas, *Cours de biologie humaine. Ver.* (2006), 07-11-2006.
- [21] F Yamamoto, H Claussen, T White, J Marken, SI Hakamori, *Nature*, (1990), 345: 229-233.
- [22] F Yamamoto, PS Neill, SI Hakamori, *Biochem. Biophys. Res. Commun.*, (1992), 187: 366-374.
- [23] F Yamamoto, *Vox Sang.*, (1995), 69: 1 7-19
- [24] Centre National de Transfusion Sanguine (CNTS) Côte D'Ivoire, *Rapport d'activité* (2008), 2007 ; 91 p.
- [25] Anonyme *CNTS* (2011), 35p.
- [26] M Sbiti, M Bahji, H Zahid, M Rafi, M Benkirane, *La gazette de la transfusion*; (2002), 175: 4-7
- [27] F Sombo Mambo, J Seka, R Cabannes, *Revue médicale de Côte d'Ivoire*, (1987), 21 (83) :16-25.
- [28] C Dulat, JL Rey, C Trolet, *Med Afr Noire*; (1989), 36:885-890.
- [29] A Empana, A Jouvenceau, *Rev Fr Transfus Immunohematol*; (1982), 25(1):19-23.
- [30] A Loua MR Lamah, NY Haba, M Camara, *Transfus. Clin et Biol.*, (2007), 14 : 435-439
- [31] J Mwangi, *East Afr Med J*; (1999), 76 :615-628
- [32] G Garratty, SA Glynn, MC Entire, *Transfusion* ; (2004), 44 (5):703-706.
- [33] G Daniels, ME Reid, *Transfusion*; (2010), 50 (2):281-293.
- [34] N Said, F Ben Ahmed, A Doghri, E Ghazouani, S Layouni, N Gritli, *Transfus Clin Biol*; (2003), 10:331-401.

-
- [35] WM Watkins, *Transfus Med.* ; (2001), 11 :243-265.
- [36] G Blavy, D Thiam, I Aidara, L Diakhate, *Rev Fr Transfus. Immunohematol*; (1986), 29:341-405.
- [37] B Carrit, T Kemp, M Poultei, *Hum.Molec.Genet*; (1997), 6 : 843-850
- [38] B Cherif Zahar, G Mattem, Levankin, P Bailly, JP Cartion et Y Collin, *Hum. Genet.*, (1991), 86 : 398-400.
- [39] I Mouro, Y Colin, B Cherif-Zahar, JP Cartron, C Levankin, *Nature genetics*; (1993), 5: 62-65.
- [40] TJ Morgan. and WM Watkins, *specifity.Glycoconjugate Journal*, (2001), 17: 501-530
- [41] L Castilho, G Daniels, WA Flegel, G Garratty, CL Francis, JM Moulds, JJ Moulds, ML Olsson, J Poole, *Berlin report. Vox Sang*; (2011), 101(1):77-82.
- [42] AG Falusi, OG Ademowo, CA Latunji, A Okeke, *Afr J Med. Sci.*, (2000), 29: 23-26
- [43] LL Cavalli-Sforza, P Menozzi, A Piazza, *Princeton University Press*. (1994), p10
- [44] J Chiaroni, *Aix. Marseille II. Faculté de Médecine* ; 2003, p12