Original Article

# *RHD* Gene Polymorphism among Palestinians of Gaza Strip.

**Dawoud M.I.[1], Shubair M.E.[2], Sharif F.A.[2*]**
1. Al-Shifa Hospital, Central Blood Bank, Gaza. 2. Medical Technology Department, Faculty of Science-Islamic University, Gaza.

# الأنماط الجينية للعامل الريزيسي في قطاع غزة – فلسطين

## الملخص

يعتبر العامل الريزيسي من أهم وأكثر الفصائل الدموية تعقيدا، حيث يحتوي هذا النظام على عدد كبير من الأنتيجينات التي هي عبارة عن مواد بروتينية تخلق بواسطة زوج من المورثات الجينية المتشابهة، والموجودة على الكروموسوم رقم 1، إحدى المورثات *RHCE* تنتج أربعة أنتجينات بينما المورثة الأخرى *RHD* تنتج أنتجين D والذي يعتبر الأساس في هذا النظام وإليه تعزى فصيلة الدم السالبة والموجبة.

الأكثر أهمية وتنوعا وله أهمية طبية بالغة في مجالات نقل الدم فهو ذو قدرة عالية RhDويعد على إحداث رد فعل مناعي لتكوين أجسام مضادة وبتركيز عال، والتي تعد السبب الرئيس في مرض تحلل الدم عند الأطفال حديثي الولادة والكثير من حالات عدم التطابق أثناء عملية نقل الدم.

الفصيلة السالبة للدم عادة ما تنتج عن غياب أو إزالة كنتيجة لخلل في المورثة، والتي تنجم إما عن Rh في نظام RHC و RHDطفرة أحادية في المورثة أو حدوث عملية تهجين بين كلا المورثتين والتي يمكن Anti-D لها القدرة على حث جهاز المناعة لتكوين أجسام مضادة Partial D. فصيلة أن تسبب تحللا للدم المنقول كما أنها تسبب مرض تحلل الدم عند الأطفال حديثي الولادة.

هناك نوعان واسعا الانتشار من Partial D ويطلق عليها Dvi و DNB ولهما أهمية طبية بالغة في مجال نقل الدم ومرض تحلل الدم لدى الأطفال حديثي الولادة.

في هذه الدراسة قمنا بتحليل الحمض النووي DNA والمستخلص من 102 عينة دم من المتبرعين بالدم والذين يحمل بعضهم الفصيلة الموجبة وبعضهم الآخر الفصيلة السالبة، وأجرينا فحوصات مسحية على كافة العينات لتحديد Dvi و DNB بين هؤلاء المتبرعين باستعمال تقنيات البيلوجيا الجزيئية PCR المتطورة، وكانت النتائج وجود 3 متبرعين يحملون النوع الأول و3 متبرعين يحملون النوع الثاني.

كذلك اشتملت الدراسة على تحديد البنية الوراثية للعينات ذات الفصائل السالبة لمجموعة المتبرعين بالدم الذين شملوا بالدراسة، ووجد أنها في الأغلب ناجمة عن إزالة كاملة للمورثة الجينية RHD. كما أشارت نتيجة البحث إلى وجود تطابق كامل بين الفحوصات المصلية وفحص النووي في العينات ذات الفصائل الموجبة.

---

الكلمات المفتاحية: النظام الريزيسي، العامل الريزيسي D ; Dvi ; DNB ; Anti-D والمورثة الهجينة، PCR-SSP.

**Abstract**

Rh system is one of the highly complex blood group systems with many serologically defined Rh antigens. These antigens are expressed by proteins encoded by a pair of highly homologous genes located on chromosome 1. *RHCE* gene encodes the CcEe antigens, while the *RHD* encodes the D antigen. RhD is the most important, immunogenic and polymorphic Rh antigen from the clinical aspects (comprising at least 30 epitopes), as it plays a key role in transfusion medicine. Anti-D antibodies remain the leading cause of the hemolytic disease of the newborn (HDN), and antigen D compatible transfusion is a standard practice in transfusion therapy. Partial D lacks one or more D epitopes, and a partial D individual may be immunized on exposure to a normal D positive during blood transfusion or pregnancy. The $D^{VI}$ and DNB variants are the most frequent partial Ds that lack some D epitopes, $D^{VI}$ is usually typed as D negative while DNB is typed as D positive.

We have examined 102 genomic DNA samples collected from blood donors expressing D positive (79 samples) and negative phenotypes (23 samples), to detect $D^{VI}$ and DNB variants, and to investigate the molecular basis of Rh negative phenotype. To verify the $D^{VI}$ variant; simplex PCR was used to detect the presence or absence of *RHD* exon 10/intron 4, while PCR-SSP was used to detect the DNB variant. Three $D^{VI}$ and three DNB samples were detected. The PCR results indicated a deletion of *RHD* gene in D negative specimens.

The results show that the frequency of the $D^{VI}$ phenotype in Palestinians is greater than expected and routine screening for this phenotype should become mandatory for equivocal weak D blood samples.

**Key words:** *RHD* gene, $D^{VI}$, DNB, Partial D.

## Introduction:

The Rh system is one of the most and highly complex blood group systems known in humans, with many serologically different Rh antigens. These antigens are expressed as part of a protein complex on the red blood cell (RBC) membrane. The expression is confined to the erythroid cell line, and therefore Rh antigens are only expressed on RBCs. The composition of the complex is unknown, but it is supposed to be a tetramer, consisting of two molecules of Rh associated glycoprotein (RhAG) and two molecules of Rh proteins. The Rh proteins may be RhD (carrying the D antigen) or RhCE (carrying the C or c antigen and the E or e antigen). It is unknown whether both RhCE and RhD can be in a single complex, but in D negative individuals the complex would only contain RhCE [1,2].

The significance of Rh system is related to the facts that: 1) The Rh antigens are highly immunogenic and of great importance for transfusion medicine; 2) The complexity of its antigens, which stems from the highly polymorphic genes that encode them; 3) The great differences among races in the frequencies of the alleles of the *RH* gene complex. According to those facts, the Rh system remains the most

polymorphic and immunogenic blood group system known in humans [1].

The D antigen is the most important Rh antigen consisting of at least 30 epitopes. Partial D phenotypes occur when there is absence of one or more of these epitopes. The $D^{VI}$ phenotype is the most common partial D phenotype in Europeans, lacking most D antigen epitopes. $D^{VI}$ mothers may become immunized by transfusion with D positive blood (if typed as D positive using polyclonal typing reagents) or by fetuses which have all of the D epitopes. This situation can give rise to HDN [3].
DNB is another partial D that presents a normal D in routine typing, indicating that DNB carriers are generally able to produce strong allo-anti-D [4].

Despite the importance of the Rh antigens in blood transfusion and HDN, the function of its proteins is speculative, and may involve transporting ammonium ions across the RBC membrane and maintaining the integrity of the RBC membrane. Substitutions of amino acids that are located in Rh transmembraneous segments, may affect the function of the Rh protein [1]. New evidence, however, suggest that the RhAG possesses a channel for passage of neutral gases, and that D and CE polypeptides are unlikely to have a transport function [5].

Hitherto, no previous studies were conducted in the field of blood transfusion medicine and its adverse reactions in Gaza Strip, Palestine. RhD typing discrepancies of blood donors, patients, mothers and babies which complicate the transfusion process are not uncommon. To detect and characterize the more frequent partial Ds namely, $D^{VI}$ and DNB, and to identify the molecular basis of Rh negative phenotype, PCR-SSP and monoplex PCR were used to screen 102 positive and negative RhD blood donors` specimens.

**Materials and Methods:**

**Study design:**
This descriptive study was designed to detect the D variants that are frequent in other ethnic groups (namely; $D^{VI}$ and DNB) and to determine the molecular basis of RhD negative phenotype among a group of blood donors residing in Gaza Strip.
The detection of $D^{VI}$ variant is based on exploiting the differences between *RHD* and *RHCE* genes, where, *RHCE* has a larger intron 4, whereas the *RHD* gene has a larger exon 10. $D^{VI}$ phenotype is characterized by absence of *RHD* intron 4. Thus, the presence of *RHD* exon 10 with *RHCE* intron 4 along with absence of *RHD* intron 4 is a strong indicator for the existence of the RH *D/CE/D* hybrid allele and consequently the presence of $D^{VI}$ phenotype [6].
Amplification of both *RHD* exon 10/ intron 4 is also used for investigating the molecular basis that generates the Rh negative phenotype. To define the DNB variant, PCR-SSP was used to detect the point mutation in *RHD* exon 7 that causes G355S substitution [4,7].

**Blood samples:**
The D negative and D positive blood samples used in this study were collected at random from 102 unrelated Palestinian blood donors: 79 RhD positive and 23 RhD negative. Ninety blood donor samples were obtained from Al Shifa hospital Central Blood Bank, and 12 blood donor samples were obtained from the Central Blood Bank Society.

**Ethical Considerations:**
The local ethics committee (Helsinki Committee) approved the study protocol.

**Serological RhD typing:**
Phenotyping of the RhD for all samples was performed in blood bank centers using commercial monoclonal anti-D (LORNE Laboratories, UK). Blood samples were serologically typed for RhD by the method of direct hemagglutination test according to the manufacturer protocol.

**Molecular analysis:**

**DNA Extraction:**
DNA was extracted from blood samples using Wizard® Genomic DNA purification Kit (Promega Corporation, Madison, USA). Extraction was done following the manufacturer instructions.

**$D^{VI}$ Detection by PCR:**
All D positive and D negative genomic DNAs were examined for $D^{VI}$. The method is based on analyzing two *RHD* regions by using two separate monoplex PCR assays. Briefly, the assay involved the amplification of *RHD* exon 10/ intron 4, and *RHCE* intron 4, the latter was used as an internal control. The first region that was subjected for PCR investigation was the *RH* intron 4. Specific primers; 4-F (CGATACCCAGTTTGTCTGCC) and 4-R (AGAACATCCACAAGAAGAGGG) were used to distinguish and identify intron 4 of both *RHD* and *RHCE*. The two *RHD* and *RHCE* intron 4 PCR fragments were obtained from the same primer pair, when the primers paired with the *RHD* intron 4, it amplified a product of 478 bp, whereas, when paired with the *RHCE* intron 4, a product of 1126 bp was produced. Each PCR run was performed with necessary negative controls [8].

The second target region that was subjected to PCR was *RHD* exon 10. The specific primers; 10-F (TTGGATTTTAAGCAAAAGCATCC ) and exon 10-R (ATTCTCCTCAAA GAGTGGCAG) were applied to amplify and detect a sequence located between *RHD* specific 3' untranslated region (UTR) and *RHD* exon 10, which produced a product of 185 bp [8].

The two separate PCRs were performed in similar conditions and carried out in 0.2 ml PCR microfuge tubes with a final volume of 20µl, containing, 2 µl of 2.0 µmol of each specific primer, 10 µl of PCR Mater Mix (Promega Madison, USA), 2.0 µl of template genomic DNA (100-200 ng), 4.0 µl of nuclease free ultra-pure water. The following profile was used for both RHD exon 10 and *RHD*/*RHCE* intron 4 PCR assays. After an initial denaturation for 10 minutes at 94°C, the samples were subjected to 30 cycles of PCR in a DNA thermal cycler (Eppendorf, Germany). Each cycle consisted of: 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1.5 minutes, followed by a final extension at 72°C for 5 minutes. Equal volumes of the *RH* intron 4 and *RHD* exon 10 PCR products were mixed and analyzed by electrophoresis on 1.5 % agarose gels containing 10 µg/ml ethidium bromide.

**PCR-SSP for DNB Detection:**
A PCR with sequence specific priming (PCR-SSP) was used to detect or to confirm the 1063G→A substitution in the *RHD* exon 7 (DNB allele). DNB specific sense primer re77 (TCTCCACAGCTCCATCATGGG) and antisense primer DNBb (CAGTGACCCAC ATGCCATTACT) at a concentration of 2.0 µmol were

used to detect the specific mutation in *RHD* exon-7. PCR should amplify a fragment with 118 bp product across *RHD* exon 7 [4,7].
Human growth hormone (HGH) gene was used as a positive control where a 434 bp fragment was co-amplified using the primers (hgh-F) (tgccttcccaaccattc cctta) (intron/intron) and (hgh-R) (ccactcacggatttctgttgtgtttc).
Amplification was carried out in 0.2 ml PCR microfuge tube with a final volume of 20 µl, each containing 2 µl of 2.0 µmol DNB primers (DNBre77, DNBb), 0.5 µl of 2.0 µmol HGH primers (hgh-F, hgh-R), 10 µl of PCR Mater Mix (Promega Madison, USA), 1.5 µl of template DNA (100-200ng), 3.5 µl of nuclease free ultra-pure water. Each PCR run was performed with a negative control without a DNA template.
Thirty two PCR cycles were performed on the DNA consisting of an initial denaturation of 2 min at 94°C, followed by ten cycles of 10 seconds denaturation at 94°C, 1 min annealing/extension at 65°C, and 30 sec at 72°C followed by 22 cycles of 30 sec denaturation at 94°C, 1 min annealing at 61°C, 30 sec extension at 72°C and a final extension of 4 min at 72°C. The PCR products were then analyzed by electrophoresis as described above.

**Statistical Analysis:**
z test for two proportions, at 95% confidence level, was applied to test for significant difference between the Dvi frequency observed in this study and that reported for Caucasians.

**Results:**

**Immunohematology:**
All the 102 donor blood samples were serotyped for RhD using commercial MoAb. Seventy nine samples were D positive and 23 were D negative.

**Molecular screening for Rh $D^{VI}$ :**
Detection of the $D^{VI}$ alleles depends on the absence of *RHD* intron 4 with concomitant presence of *RHD* exon 10. The $D^{VI}$ phenotype was verified by using two independent PCRs: one PCR coamplifies introns 4 of the *RHD* and *RHCE* genes, and produces two products, a 478 bp corresponding to *RHD* intron 4 and a 1126 bp from *RHCE* intron 4 (was also used as an internal control) from D positive genomes (two bands were detected). The $D^{VI}$ which is characterized by absence of *RHD* intron 4 (should yield only one 1126 bp fragment) and the same applies for RhD negative specimens. The second PCR primer pair is specific for exon 10 of the *RHD* gene only, and amplifies a product of 185 bp in D positive and $D^{VI}$ but not in D negative samples.

Three patterns of PCR products were apparent when the mixture of the two PCR products was analyzed: 1) Detection of both *RHD* intron 4/ exon10 regions. 2) Absence of both *RHD* regions, 3) Presence of *RHD* exon 10, but absence of *RHD* intron 4. Of the 102 blood donors tested, 79 samples (77.5%) had both regions of *RHD*, 20 samples (19.6%) lacked both *RHD* regions and 3 samples (2.9%) had *RHD* exon 10 only (Figure 1). *RHCE* intron 4 was detected in all samples.

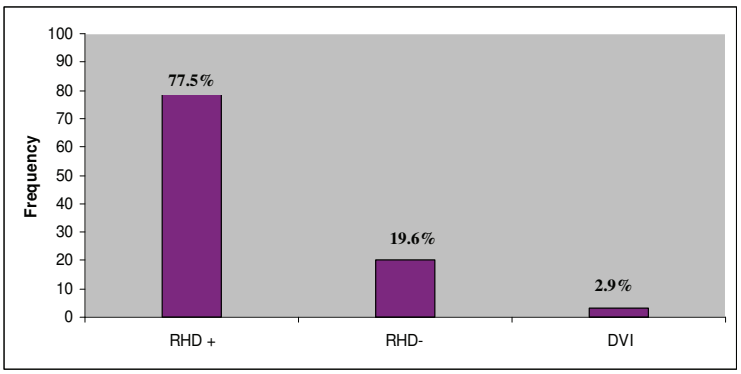


**Figure 1**. Frequency and percentage of PCR patterns in D+/- blood donor samples

**Screening D positive donor samples for *RHD* exon 10 and intron 4:**

The first pattern was observed in RhD positive blood donors. When we screened the 79 serological RhD positive samples, all samples (100%) showed the presence of both introns 4 and exon 10 of *RHD* gene. Intron 4 of *RHCE* gene was always detected and indicated the success of PCR amplification (Figure 2).

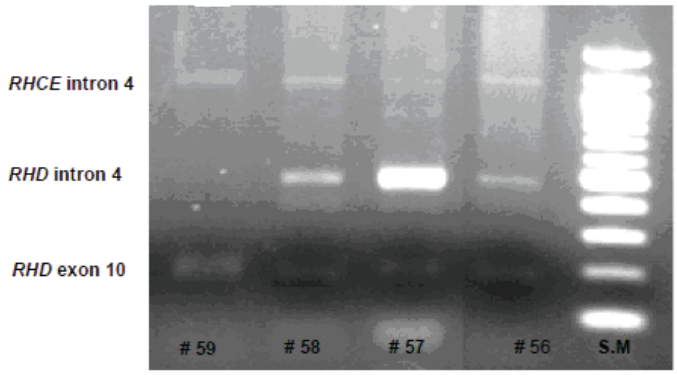


**Figure 2.** A representative photograph of PCR results of $D^{vi}$ and D positive samples. Samples #56, 57 and 58 represent D positive and sample #59 indicates a $D^{vi}$ sample. S.M is the size marker.

**RhD negative:**

Of the 23 D negative blood specimens examined, twenty D negative (87%) lacked both *RHD* regions but retained the *RHCE* intron 4 band (the internal control). The specific primers could not recognize the presumably deleted sequences of *RHD* gene.

**$D^{VI}$ variant:**

The assay mentioned above was discordant in three D negative phenotypes. Of the 23 D negative samples 3 (13%) had *RHD* exon 10 only, suggestive for a $D^{VI}$ variant. See Figure 2 for representative results.

**Statistical Analysis:**

z test for two proportions (with a z value of 9.63) revealed a highly significant difference between Dvi frequency observed in this study (2.9%) and that reported for Caucasians (0.02%).

**Molecular screening for DNB:**
All the 79 D-positive blood samples were examined for DNB variant. The PCR-SSP amplified a product of 118 bp across *RHD* exon-7 in 3 of the 79 D-positive, and these were considered as DNB variants. The rest of the samples yield no product indicating absence of G355S substitution. The 434 bp product of human growth hormone gene *(HGH)* which served as an internal control was evident in all amplifications (Figure 3).

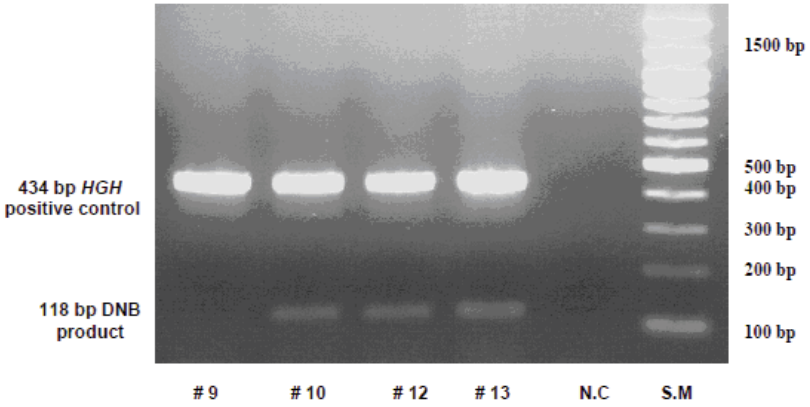


**Figure 3**. A photograph illustrating PCR-SSP results for detection of DNB allele. Samples # 10, 12 and 13 represent positive DNB results. Sample # 9 is negative for DNB. HGH represents the control 434 bp product of human growth hormone gene. S.M is the size marker.

**Discussion:**

The current study tested the correlation between the serological phenotype of Rh blood group and the molecular structure of the *RHD* gene and the relevant alleles.

In individuals expressing normal D phenotype a full concordance was observed between serological typing and presence of an intact *RHD* gene, where the two usually examined *RHD* regions (exon 10 and intron 4) were detected by PCR assay.

The frequency of D positive/negative is not yet estimated in the Palestinians residing in Gaza strip, but in Palestinians residing in Kuwait the D positive phenotype was reported as 92% [9]. In other ethnic groups like Caucasians, the D positive frequency was found to be about 82%-85%, in Africans 93%-97% and about 99% in the Far East populations [2,10,11].

The D negative phenotype is generally due to homozygosity of *RHD* gene deletion. Our results showed that, of the 23 serologically D negative donor samples, 20 D negative samples (87%) were completely concordant with both exon 10 and intron 4 bands absence pattern, which according to many authors indicates a gross deletion of the *RHD* gene [1,10,12]. PCR analysis for the rest of the *RHD* gene exons/introns and DNA sequencing are needed in order to support this conclusion.
The examination of *RHD* intron 4 and exon10 of negative samples by using the specific primers excluded the confusion of either *RHD* pseudogene *($RHD^{\Psi}$)* or any other unexpected molecular variants with grossly intact *RHD* genes. This result supports the hypothesis that, the majority of D

negative genomes examined, appear to lack all portions of the *RHD* gene. In D negative African Americans and South African people of mixed race, 54% of African Americans and 81% of South African donors of mixed race were shown to have no *RHD* gene [13].

Other workers have described variant D negative English and Scottish individuals with an apparently intact *RHD* gene, and intact *RHD* transcripts. The molecular basis was explained by a point mutation in the *RHD* gene at codon 41 generating a premature stop codon. This pattern was also shown in Japan, where intact *RHD* gene was found in 27.7% of the D negative Japanese donors. Still, other investigators found a 37 bp insertion in *RHD* exon 4 introducing a premature stop codon at position 210. Two-thirds of D negative Africans were found to carry this mutant *RHD* allele [11,14].

$D^{VI}$ phenotype is the most common among Europeans and the most clinically significant partial D phenotype in transfusion medicine. It lacks most epitopes compared to any other D variant, with very low antigen density. Among the D variants, $D^{VI}$ is still considered as the major cause of allo-immunization with anti D [2,3,15,16]. Of the 23 D negative blood donors investigated in this study, the PCR assay results of 3 samples (13%) proved the absence of *RHD* intron 4, and these were considered as $D^{VI}$ variant.

Many investigators confirmed that all *RHD* genes in individuals expressing the $D^{VI}$ phenotype lack intron 4 of the *RHD* gene. A hybrid *RHD-RHCE-RHD* gene, resulting from a probable gene conversion event in which the complete *RHD* gene exons 4, 5, and 6 are replaced by the corresponding exons of the *RHCE* gene is the most accepted mechanism for generating this $D^{VI}$ phenotype [2,3,11].

The negative phenotype of the $D^{VI}$ cannot be explained by the lack of the RhD epitopes alone, but may be also due to a reduced number of RhD proteins accessible on the RBCs' surface. The four known $D^{VI}$ types (I, II, III, IV) have the same clinical significance, because all types share *RHCE* exons 4 and 5 and their negative reaction patterns don't differ with polyclonal and most monoclonal antibodies and all four types can readily become allo-immunized when encountering conventional D [2,3,6].
The frequency of $D^{VI}$ variant differs among populations, the phenotype has a frequency ranging between 0.02% to 0.05% in Caucasians, about 0.02% in Germany, and 0.04% in the English people [15,16]. The results of our study show that the frequency of the $D^{VI}$ phenotype in Palestinian, and probably in Arab populations, may be greater than expected. Moreover, statistical analysis revealed that the observed frequency (2.9%) is significantly different from that reported for Caucasians, a result suggesting differences in the genetic composition of the two populations.

Several partial D antigens permit anti-D immunization in their carriers and DNB variant is one of these. In routine D typing, DNB samples are usually typed as positive RhD and are agglutinated by most anti-Ds, including almost all commercial anti-D typing reagents.

Although the DNB variant lacks 4 epitopes (epD6 and epD31 as well as part of epD18 and epD23), the three

samples observed in this study were serologically typed as D positive without noticeable weakening of the antigen D reaction. Of the 79 D-positive donors, 3 (3.8%) were positive with PCR-SSP. This finding confirms one of the mechanisms that generate the partial D phenotype, where a point mutation in the exofacial loop 6 characterizes the DNB features and classifies it as a partial D [4,12].

The application of molecular approaches in determining the genetic polymorphisms of *RHD*, their frequencies and distribution in the population have critical importance for the practical application of blood transfusion, for example, *RHD* PCR may allow the identification of D positive blood units missed by routine serology. In instances where $D^{VI}$ phenotype blood donors are identified as D negative by use of inappropriate D typing reagents, the recipient will be at risk of alloimmunization, so appropriate anti-D reagent must be used to type the $D^{VI}$ blood donor as a D positive. On the other hand, $D^{VI}$ recipient should be treated and typed as D negative, triggering D negative transfusions, which is the clinically favored management. Because the frequency of partial D is low, the D negative transfusion would not compromise the D negative blood supply. Similarly, when $D^{VI}$ phenotype mothers are identified as D positive by use of inappropriate D typing reagents (e.g, polyclonal anti-D), $D^{VI}$ mothers may become alloimmunized against their normal D positive infants where prophylactic anti-D is not administered [2,4,7,16]. In cases where alloimmunization has occurred prenatal detection of a fetus carrying a normal *RHD* gene can be achieved by PCR analysis of fetal DNA [17].

Today, $D^{VI}$ may be detected specifically by suitable combinations of monoclonal anti-D antibodies. This strategy became mandatory in Germany since 1996, and in the United Kingdom as well, where, D typing reagents are adopted to type $D^{VI}$ patients and mothers as D negative. All commercial monoclonal anti-Ds reagents that don't bind $D^{VI}$ can agglutinate DNB, therefore, a serologic strategy for detecting DNB would have to rely on a separate anti D that discriminates DNB from normal D. D negative transfusion strategy for DNB may be advantageous.

**Conclusion :**

The present study revealed that the D negative phenotype is common in our population and is largely due to complete deletion in the RHD gene. Two partial D variants namely DNB and $D^{VI}$ were detected and these should be considered in refining the transfusion strategy in our country. Further work is needed to unravel the allelic diversity of RHD gene in the Palestinian population and other Arab nations.

**References:**

1. Avent ND, Reid ME. The Rh blood group system. Blood 2000;95(2):375-387.

2. Wagner FF, Gassner C, Müller TH, Schönitzer D, Schunter F, Flegel WA. Three molecular structures cause Rhesus D category VI phenotypes with distinct immunohematologic features. Blood 1998;91(6):2157-2168.

3. Avent ND**,** Liu W**,** Jones JW**,** Scott ML**,** Voak D**,** Pisacka M, Watt J, Fletcher A. Molecular analysis of Rh transcripts and polypeptides from individuals expressing the DVI variant

phenotype: An RHD gene deletion event does not generate all DVIccEe phenotypes. Blood 1997;89(5): 1779-1786.

4. Wagner FF**,** Eicher NI**,** Jørgensen JR**,** Lonicer CB**,** Flegel WA. DNB a partial D with anti D frequent in Central Europe. Blood 2002; 100(6):2253-2256.

5. Burton NM and Anstee DJ. Structure and significance of Rh proteins in red cells. Current Opinion in Hematology 2008; 15(6):625-630.

6. Mouro I, Le Van Kim C, Rouillac C, van Rhenen DJ, Le Pennec PY, Bailly P, Cartron JP, Colin Y. Rearrangements of the blood group RhD gene associated with the DVI category phenotype. Blood 1994; 83 (4):1129-1135.

7. Chen Q. Random survey for *RHD* alleles among D positive Europeans [dissertation]. Ph.D. Thesis. Ulm university-Germany; 2004,

8. Qun X, Jianye Z, Qinyou W, Shixun Z, Guiling SI. RHD gene polymorphism among RhD-negative Han Chinese. Chinese Medical Journal 2003;116(10):1539-1543.

9. Mourant AE, Kopec AC, Domaniewska-Sobczak K. The distribution of the human blood groups and other polymorphisms. 2nd ed. London, England; Oxford University Press, 1976.

10. Wagner FF, Flegel WA. *RHD* gene deletion occurred in the *Rhesus box.* Blood 2000; 95(12):3662-3668.

11. Okuda H, Kawano M, Iwamoto S, Tanaka M, Seno T, Okubo Y and Kajii E. The *RHD* gene is highly detectable in RhD negative Japanese donors. Journal of Clinical Investigation 1997; 100(2):373.379.

12. Flegel WA, Wagner FF, Muller TH, Gassne C. Rh phenotype prediction by DNA typing and its application to practice. Transfusion Medicine 1998; 8:281.302.

13. Singleton BK, Green CA, Avent ND, Martin PG, Smart E**,** Daka A**,** *et al.* The presence of an *RHD* pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. Blood 2000; 95(1):12-18.

14. Eid SS. Molecular basis of RhD-positive/D-negative chimerism in two patients. Eastern Mediterranean Health Journal 2004; 10(1):228. 241.

15. Wagner FF, Kasulke D, Kerowgan M, Flegel W A. Frequencies of the blood groups ABO, Rhesus, D category VI, Kell, and of clinically relevant high-frequency antigens in South-Western Germany. Infusionsther Transfusionsmed 1995; 22:285.290.

16. Esteban R, Montero R, Flegel WA, Wagner FF, Subirana L, Parra R, *et al.* The D category VI type 4 allele is prevalent in the Spanish population. Transfusion Medicine 2006; 46:616-623.

17. Bennett PR, Le Van Kim C, Colin Y, Warwick R, Zahar BC, Fisk NM, *et al*. Prenatal determination of fetal RhD type by DNA amplification. The New England Journal of Medicine 1993; 329(9):607-610.

***Corresponding Author:** Prof. Fadel A. Sharif, Medical Technology Department, Faculty of Science-Islamic University, Gaza. Tel: +970 8 2860700 ext. 2675. Fax: +970 8 2860800. E-mail: fsharif@iugaza.edu.ps .