INVESTIGATING THE GENETIC EFFECT OF VKORC1 GENE POLYMORPHISM ON WARFARIN RESPONSE IN EGYPTIAN HEART PATIENTS

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Warfarin is a common oral anticoagulant, but the dose needed for each patient can vary by as much as 20 times due to both environmental and genetic factors. Some of the most important genetic targets of warfarin include the vitamin K epoxide reductase complex 1 (VKORC1) gene. This study investigated the genetic effect of the VKORC1 gene on warfarin response using real-time PCR in a total of 100 warfarin-treated Egyptian heart patients and 40 controls. This study confirmed a genetic association of the VKORC1 SNP rs9934438 with warfarin response. The VKORC1 SNP analysis identified the presence of 26% homozygous variant (A/A), 42% heterozygous variant (A/G), and 32% wild-type variant (G/G) among patients compared to 20%, 25%, and 55% of controls. A significant difference was found between VKORC1 genotype allelic distribution in study patients and controls (P<0.05). Patients carriers of the VKORC1 genotype (A/G) required a lower daily warfarin dose than the other variants (P=0.006). The total number of patients who reached the optimal therapeutic goal of warfarin dose of 100 study patients was 19 compared to 81 ones who did not. Females required lower daily warfarin doses than males. VKORC1 gene polymorphism did not affect INR outcomes, but it was associated with a decrease in warfarin response. The VKORC1 gene polymorphism could explain the interindividual variation in warfarin response among Egyptian patients. Our findings suggest that dosing algorithms incorporating the genetic elements of the VKORC1 genotype are essential to determine warfarin dose and could optimize the therapeutic effectiveness of warfarin and minimize its adverse effects.

Keywords: Warfarin, Anticoagulation, Pharmacogenomics, VKORC1, Genotyping, Egyptians

INTRODUCTION

Cardiovascular illnesses¹ include, but are not limited to, coronary artery disease, stroke, peripheral artery disease, and heart failure. When a blood clot develops in a vein or an artery, it blocks blood flow and causes symptoms².

Anticoagulants are chemicals that reduce or halt blood coagulation³. Warfarin, an oral anticoagulant, is widely used for the management of a wide range of thromboembolic diseases⁴. Warfarin is an anticoagulant that works by preventing the liver from producing vitamin K-dependent clotting factors II, VII, IX, and proteins C and S. It is a racemic combination of R- and S-enantiomers.

Warfarin is notoriously difficult to dose properly because of its small therapeutic window and large individual variation. The effectiveness of warfarin and the frequency of dosing required to avoid adverse effects may be affected by a variety of factors. In addition to demographic and environmental influences,
genetic variants have been shown to significantly impact warfarin dose.\textsuperscript{6,7} There was a robust correlation between warfarin responsiveness and dosage algorithms\textsuperscript{8,9} and genetic variants in genes controlling warfarin metabolism and pharmacodynamic response. Vitamin K epoxide reductase complex 1 (VKORC1) and cytochrome P450, subfamily 2, polypeptide 9 (CYP2C9) are examples of such genes. Variation in CYP2C9 genotype accounts for around 10\% of the observed dosage range\textsuperscript{9,10} for therapeutic warfarin. Warfarin response variability may be due in part to polymorphisms in the gene encoding VKORC1, which have been demonstrated to account for around 30\% of the variability of warfarin dosage\textsuperscript{11,12}. Vitamin K epoxide reductase (VKORC1) is a protein that is encoded by the VKORC1 gene\textsuperscript{13,14}. Warfarin is most effective when dosed according to an individual's pharmacogenetic profile\textsuperscript{15}, and anticoagulation levels should be closely monitored during treatment. Several studies\textsuperscript{16-21} shown that CYP2C9 and VKORC1 polymorphisms substantially impacted warfarin responsiveness and clinical outcomes. Differences in warfarin dosage and clinical outcomes were shown to be associated with polymorphisms in the VKORC1 gene in the Egyptian population\textsuperscript{22,26}. We are interested in the VKORC1 gene because of its potential involvement in vitamin K deficient illnesses and its relevance in the large interpatient variability in warfarin dosing. Therefore, it seems that the VKORC1 genotype may be used as a reliable predictor of the optimal warfarin dosage. The study's goals were to determine whether VKORC1 gene polymorphisms influence warfarin response in the Egyptian cardiac population and to identify those whose inadequate response to warfarin therapy is due to VKORC1 gene abnormalities.

**PATIENTS AND METHODS**

**Study design and patients**

At Assiut University Cardiology Hospital in Assiut, Egypt, 100 patients who were receiving warfarin and 40 controls who were not receiving warfarin participated in a prospective research. Participants in this trial began taking warfarin between March 2020 and February 2021. Patients were only considered if they were under the age of 18 when they first started taking warfarin for a period of three months. Patients with hepatic disease whose alanine aminotransferase or aspartate aminotransferase levels were greater than three times the upper limit of normal; patients with thyroid disease (hypothyroidism or hyperthyroidism); patients with renal disease; patients with malnutrition; patients who were also alcoholics or smokers; patients taking drugs that interfered with the effectiveness of warfarin; and patients who had lost clinical data during the follow-up period were excluded. This research was approved by the local ethics committee at the School of Medicine at Assiut University (research Protocol Number: 17101365). All research participants provided written informed permission.

**Data collection and follow-up**

The patients' sexe, age range, weight, body mass index, blood glucose, liver and kidney function, blood gases, blood pressure, and dietary habits were recorded. All of the patients' clinical features, including their warfarin dosage, reason for treatment, INR measurement, lipid profile (cholesterol, LDL, and HDL), comorbidities, and other drugs, and drug interactions were taken into account.

**Blood sample**

When taking blood for routine laboratory investigations, a sterile venipuncture was used to collect 5 mL from each patient; 2 mL was placed in a 3.2\% buffered sodium citrate solution for the measurement of prothrombin time (PT), prothrombin concentration (PC), and international normalised ratio (INR); and 3 mL was placed in an EDTA tube and frozen at -70°C for DNA extraction.

**Prothrombin time and international normalized ratio measurement**

Measurement of prothrombin time (PT) was performed using (photo-optical) fully automated SYMEX CA-1500 System USA 4.00 Compact® Hemostasis System. An aliquot of platelet-poor plasma was incubated at 37°C with a reagent containing a tissue factor, phospholipid thromboplastin, and CaCl2. In order to get the International Normalized Ratio
(INR), the PT ratio must be adjusted. The INR value is equal to the patient's PT divided by the standard PT multiplied by the thromboplatin's International Sensitivity Index (ISI).

**DNA extraction**

Using the QuiQueb DNA mini extraction kit (Qiagen, Germany), we isolated DNA from patient peripheral blood samples as per the manufacturer's instructions. Until VKORC1 genotyping, the DNA was stored at -20 degrees Celsius.

**SNP selection and genotyping**

We isolated DNA from peripheral blood samples of our patients by following the manufacturer's instructions for the QuiQueb DNA mini extraction kit (Qiagen, Germany). The DNA was kept at -20 degrees Celsius until the VKORC1 genotyping was completed.

**Clinical Outcomes**

The influence of CYP2C9 and VKORC1 polymorphisms on anticoagulation status and patient improvement were assessed after warfarin therapy.

**Statistical analysis**

MedCalc 14 (MedCalc software bvba, Ostend, Belgium), XLSTAT Version2014.5.03 (Addinsoft, Massachusetts, The United States), and IBM SPSS Statistics 17 (IBM Corp., Armonk, New York, United States) were used for statistical analysis. Quantitative (percentile) and qualitative (chi-squared and Fisher's exact test) data were used to compare the two groups. Statistical significance was assumed at the (0.05) level, and strong significance at the (0.01) level.

**RESULTS AND DISCUSSION**

**Results**

**Patients' demographics and clinical characteristics**

MedCalc 14 (MedCalc software bvba, Ostend, Belgium), XLSTAT Version2014.5.03 (Addinsoft, Massachusetts, The United States), and IBM SPSS Statistics 17 (IBM Corp., Armonk, New York, United States) were used for statistical analysis. Quantitative (percentile) and qualitative (chi-squared and Fisher's exact test) data were used to compare the two groups. Statistical significance was assumed at the (0.05) level, and strong significance at the (0.01) level.

**Prothrombin time (PT) and international normalized ratio (INR) Levels**

The levels of PT and INR in the study patients and controls were measured at the initiation of treatment and at the end of treatment for at least 45 days. The mean value of PT in the study patients was 21.72±5.91 compared to controls (12.1±0.81), with a highly significant difference (P<0.000). The mean value of INR was 2.31±0.11 compared to controls (1.01±0.012) with a significant difference (P<0.01), as shown in Table 1.

**Allelic frequencies distribution of VKORC1 genotypes**

The variants of the genotypes of the VKORC1 SNP rs9934438; homozygous variant (A/A), heterozygous variant (A/G), and wild type (G/G) were analyzed in both study patients and control groups. The percent of study patients carrying the homozygous variant (A/A) was 26% versus 20% in the control group; percent of the study patients carrying the heterozygous variant (A/G) was 42% versus 25% in the control group, and percent of study patients carrying the wild type variant (G/G) was 32% versus 55% in the control group, with a highly significant difference (P<0.0001) for the VKORC1 genotype variants between the study patients and control groups; as shown in Table 2 and Fig. (1). The distribution of VKORC1 genotypes (A/A, A/G, and G/G) among the study patients (N=100) was according to the Hardy-Weinberg Equilibrium (HWE), where P=0.12 and the minimal allele frequency was 0.47. However, the distribution of VKORC1 genotypes (A/A, A/G, and G/G) among the control group (N=40) was not according to the Hardy-Weinberg Equilibrium, where P <0.0001 and the minimal allele frequency was 0.26. A representative allelic discrimination plot of the VKORC1 genotyping
run is shown in Fig. 2. Table 3 shows the relation of the VKORC1 genotype with INR levels in the study and control groups.

Table 1: Demographic and clinical characteristics of the study patients and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group (N=100)</th>
<th>Control group (N=40)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (Mean±SD)</td>
<td>0.60±11.27</td>
<td>39.67±12.70</td>
<td>P= 0.347</td>
</tr>
<tr>
<td>Gender distribution (N%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45(45.0%)</td>
<td>21(52.5%)</td>
<td>P= 0.209</td>
</tr>
<tr>
<td>Female</td>
<td>55(55.0%)</td>
<td>19(47.5%)</td>
<td></td>
</tr>
<tr>
<td>PT (Mean±SD)</td>
<td>21.72±5.91</td>
<td>12.1±0.81</td>
<td>P&lt; 0.000***</td>
</tr>
<tr>
<td>INR (Mean±SD)</td>
<td>2.31±0.11</td>
<td>1.01±0.012</td>
<td>P&lt; 0.000***</td>
</tr>
</tbody>
</table>

Table 2: Allelic frequencies distribution for VKORC1 SNP rs9934438 among the the study and control groups.

<table>
<thead>
<tr>
<th>VKORC1 genotype</th>
<th>Study group (N=100)</th>
<th>P value HWE</th>
<th>Control group (N=40)</th>
<th>P value HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>26(26.0%)</td>
<td>P=0.12</td>
<td>8(20.0%)</td>
<td>P&lt;0.0001*</td>
</tr>
<tr>
<td>A/G</td>
<td>42(42.0%)</td>
<td></td>
<td>10(25.0%)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>32(32.0%)</td>
<td></td>
<td>22(55.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Allelic frequencies distribution of VKORC1 genotype among the study and control groups.
Analysis of optimal therapeutic goal (OTG) of warfarin dose in relation to gender, age, and VKORC1 genotypes

The samples of the study group (100 patients) were analyzed for optimal therapeutic goal (OTG) of warfarin dose in relation to gender, age, and VKORC1 genotype to determine the total number of patients who reached the optimal therapeutic goal of warfarin dose was only 19 patients out of 100 patients with the mean age of 35.2 ± 10.4; of which 6(13.6%) were male patients. The total number of patients who did not reach the optimal therapeutic goal of warfarin dose was 81 out of 100 patients with the mean age of 41.88 ± 11.2; of which 38(86.4%) were male patients shown in Table (4). Regarding the VKORC1 genotype among the study patients who reached the optimal therapeutic goal of warfarin dose (N=19), 3(11.5%) patients were associated with VKORC1 genotype (A/A), 9(21.4%) patients with VKORC1 genotype (A/G), and 7(21.9%) patients with VKORC1 genotype (G/G). The total number of patients who did not reach the optimal therapeutic goal of warfarin dose (N=81), 23(88.5%) patients was associated with VKORC1 genotype (A/A), 33(78.6%) patients with VKORC1 genotype (A/G), and 25(78.1%) patients with VKORC1 genotype (G/G).

Correlation of non-genetic factors and VKORC1 genotypes on daily warfarin dose

By studying the effect of the nongenetic factors such as gender and age on VKORC1 genotyping, our results have indicated that gender than age has been affected by VKORC1 genotypes; and resulted in a change in daily warfarin dose. Males required higher daily doses of warfarin (A/A, 4.29±1.24; A/G, 4.34±1.26 and G/G, 4.6±1.61) than females (A/A, 4.16±1.54; A/G, 3.29±1.37 and G/G, 4.37±1.59). More significantly, females carriers of the VKORC1 heterozygous type (A/G) required lower daily warfarin dose than other VKORC1 genotypes (P=0.006), as shown in Table 4.
Effect of VKORC1 genotype on INR values

There was a significant difference between the mean value of INR and VKORC1 genotypes of the homozygous type (A/A), (P<0.002) and the heterozygous type (A/G), (P<0.001) among the patients in the study and control groups. A high significant difference was found between the mean value of INR in the wild type of VKORC1 genotype (G/G), (P<0.000) among the patients in the study and control groups; as shown in Table 5.

Effect of VKORC1 genotypes on daily warfarin dose requirements

The mean value of daily warfarin dose at the first visit (after 15 days) in the homozygous type (A/A) was 3.73±1.32, the heterozygous type (A/G) was 3.50±1.41, and the wild type (G/G) was 3.93±1.76, with a significant difference (P<0.03) between VKORC1 genotype and daily warfarin dose among the study group. The mean value of daily warfarin dose at the second visit (after 30 days) in the homozygous type (A/A) was 4.09±1.33, the heterozygous type (A/G) was 3.80±1.35, and the wild type (G/G) was 4.51±1.64, with a significant difference (P<0.03) between VKORC1 genotype and daily warfarin dose among the study group. The mean value of daily warfarin dose at the third visit (after 45 days) in the homozygous type (A/A) was 4.40±1.37, the heterozygous type (A/G) was 4.05±1.29, and the wild type (G/G) was 4.48±1.56, with no significant difference (P>0.05) between VKORC1 genotype and daily warfarin dose among the study group; as shown in Table 6.

Table 4: Relationship between gender, Age and VKORC1 genotype in the study group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Optimal therapeutic goal (OTG) (N=19)</th>
<th>Non optimal therapeutic goal (NO OTG) (N=81)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>6 (13.6%)</td>
<td>38 (86.4%)</td>
<td>0.306</td>
</tr>
<tr>
<td>VKORC1 genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>3 (11.5%)</td>
<td>23 (88.5%)</td>
<td>0.529</td>
</tr>
<tr>
<td>A/G</td>
<td>9 (21.4%)</td>
<td>33 (78.6%)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>7 (21.9%)</td>
<td>25 (78.1%)</td>
<td></td>
</tr>
<tr>
<td>Age (Mean±SD)</td>
<td>35.2 ± 10.4</td>
<td>41.88 ± 11.2</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Table 5: Relationship between VKORC1 genotype and INR value in the study group.

<table>
<thead>
<tr>
<th>VKORC1 genotype</th>
<th>INR value (at 15 days)</th>
<th>INR value (at 30 days)</th>
<th>INR value (at 45 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>2.11±0.65</td>
<td>2.11±0.65</td>
<td>2.54±0.77</td>
</tr>
<tr>
<td>A/G</td>
<td>2.24±0.55</td>
<td>2.24±0.55</td>
<td>2.70±0.67</td>
</tr>
<tr>
<td>G/G</td>
<td>2.19±0.66 (P=0.343)</td>
<td>2.19±0.66 (P=0.701)</td>
<td>2.60±0.72 (P=0.638)</td>
</tr>
</tbody>
</table>

Statistically significant difference (p<0.05).

Table 6: Relationship between VKORC1 genotype and daily warfarin dose in the study group.

<table>
<thead>
<tr>
<th>VKORC1 genotype</th>
<th>Warfarin dose (at 15 days)</th>
<th>Warfarin dose (at 30 days)</th>
<th>Warfarin dose (at 45 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>3.73±1.32 (P&lt;0.03*)</td>
<td>4.09±1.33</td>
<td>4.40±1.37</td>
</tr>
<tr>
<td>A/G</td>
<td>3.50±1.41</td>
<td>3.80±1.35</td>
<td>4.05±1.29</td>
</tr>
<tr>
<td>G/G</td>
<td>3.93±1.76</td>
<td>4.51±1.64 (P&lt;0.03*)</td>
<td>4.48±1.56 (P=0.388)</td>
</tr>
</tbody>
</table>

* Statistically significant difference (p<0.05)
Warfarin and clinical outcomes

It is clearly evident that a polymorphism in the VKORC1 gene was associated with an interindividual variability in the dose-anticoagulant effect of warfarin. Subsequently, patients with VKORC1 genotype polymorphism require genotype-directed warfarin therapy and warfarin dosing algorithm to increase efficacy and safety in anticoagulant treatment.

Discussion

Warfarin is the most often recommended oral anticoagulant for the prevention and treatment of thromboembolic diseases. Due to the considerable inter-individual variability in dose to achieve goal INR and anticoagulation, warfarin pharmacogenetics has been intensively explored for customised treatment. The VKORC1 and CYP2C9 genotypes came up as especially important among the several known genetic variables that impact warfarin dose. The VKORC1 gene codes for vitamin K epoxide reductase (VKORC1). Treatment algorithms included CYP2C9 and VKORC1 genotyping improved dose prediction and therapeutic effectiveness in clinical studies. VKORC1 genotype has been associated with better response to warfarin treatment and lower dosage needs.

Since polymorphisms in VKORC1 account for 20-30% of the genetic variability in response to warfarin, testing for these variants has been advocated by pharmacogenomic algorithms for warfarin prescription. The VKORC1 C6484T (rs9934438), which is in almost perfect linkage disequilibrium with the SNP C3673A (rs9923231), both of which are linked with the low dosage warfarin phenotype. G3673A and haplotypes containing this variation continue to benefit from the adoption of SNP marker C6484T. The VKORC1 intronic variation C6484T was shown to be practically in perfect concordance with rs9923231, and its presence was associated with a reduced need for warfarin.

The majority of the 81 patients who did not achieve the optimum therapeutic target dosage of warfarin had the VKORC1 A/G genotype, whereas just 19 individuals in the present research met the optimal therapeutic goal dose. It was also evident that gender was a determinant of warfarin dose in this study where women require lower doses than men with a significant difference.

Based on the findings by Al-Mahayri et al. (2019) that polymorphisms (SNPs) in VKORC1 are known to promote resistance to warfarin therapy, this research underlined the impact of VKORC1 genotypes and nongenetic variables like age and gender on warfarin dosage among Emiratis. In order to reduce the time it takes to titrate to the target dosage and the number of hospitalisations and INR checks required to attain the target dose, we can anticipate the average dose need in cardiac patients by assessing VKORC1 genetic variants. This is especially helpful in low-
Multiple pharmacogenetic studies reporting on VKORC1 gene polymorphism have shown a link between genetic variation and warfarin dose variability in Egyptians. Despite the fact that both the VKORC1 and CYP2C9 polymorphisms are key contributors to warfarin doses and may help predict adverse effects in Egyptian individuals, the VKORC1 genotype was the single most important predictor of warfarin dose.

**Conclusion**

A genetic analysis for VKORC1 gene polymorphism was associated with an interindividual variability in the dose-anticoagulant effect of warfarin. Among Egyptian cardiac patients, there was a considerable impact of VKORC1 genetic variation on warfarin dosage variability. The VKORC1 (A/A) genotype group needed a significantly lower warfarin dosage compared to the VKORC1 (A/G) and (G/G) genotype groups. Dosage modifications based on INR readings cannot be used as a proxy for detecting inherited disorders.

**Acknowledgments**

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**REFERENCES**

3. H. H. Yoo, V. S. Nunes-Nogueira and


20. L. N. Al-Eitan, A. Y. Almasri and R. H. Khasawneh, "Effects of CYP2C9 and VKORC1 polymorphisms on warfarin sensitivity and responsiveness during the stabilization phase of


دراسة التأثير الجيني لتعدد الأنواع الجيني VKORC1 لدى مرضى القلب المصريين

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الوارفارين هو أحد مضادات التخثر التي يتم تناولها لالفاف على نطاق واسع. ولكن جرعة البارفارين المطلوبة تظهر اختلافًا كبيرًا ين좌 إلى 20 ضعفا بين الأفراد بسبب العوامل البيئية والوراثية. يعد الجين فيتامين ك إليوكسيد المختزل (VKORC1) أحد الأهداف الجينية الرئيسية للوارفارين. تناولت هذه الدراسة تأثير جين VKORC1 على استجابة البارفارين باستخدام تحليل تفاعلات البوليمرز المتسلسل على عدد (200) مريض قلب مصري تم علاجهم بالوارفارين مقارنة بعدد (2) مريضاً من المجموعة المنضبطة غير معالجين بالبارفارين. أكملت الدراسة وجود ارتباط وراثي بين البارفارين واستجابة البارفارين والبارفارين، وأظهرت تحليلات الأحماض الجينية لـ جين (VKORC1 SNP rs9934438) عن وجود النسب التالية: 26% (A/G)، 24% (A/A) و 22% (G/G). في مجموعة المرضى المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين التوزيع الجيني لـ VOKRC1 من بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالية (P-value) بين المرضى من المجموعة المنضبطة، وتبين وجود فرق كبير في القيمة الاحتمالا