Genetic Variants Account for Differences in Responses in Blood Pressure and Blood Flow Values to Laryngoscopy/Intubation/Extubation

Aim: There is a variation in drug response among individuals. In this study, we determined angiotensin-converting enzyme (ACE) insertion/deletion (I/D), beta2-adrenergic receptor (ADRB2) Arg16Gly and dopamine D1 receptor (DRD1) –48 G/A polymorphisms, which are presumed to be related to the transient hypertension induced by the mechanical stimulation of laryngoscopy and tracheal intubation/extubation.

Materials and Methods: One hundred American Society of Anesthesiologists (ASA) class I patients were enrolled in this study. Heart rate and blood pressure were recorded before induction (T0), after induction (T1), during laryngoscopy/intubation (Ti) and 1, 2, 3, 4 and 5 min thereafter (Ti-1-Ti-5), before extubation (Te) and 1, 2, 3, 4 and 5 min afterwards (Te-1-Te-5).

ACE I/D, ADRB2 Arg16Gly and DRD1 -48 G/A polymorphisms were investigated by polymerase chain reaction-restriction fragment length polymorphism analyses. Data were analyzed by Repeated Measure Factorial Analysis of Variance.

Results: During laryngoscopy, intubation and extubation, blood pressure, heart rate and rate pressure product (RPP) values increased, returning to baseline within five minutes. Increases at Ti were significant for all values when compared to T0 (p<0.01, p<0.001). At Ti, genotype associations were observed between DRD1-48GG genotype and systolic and diastolic blood pressures and also between heart rate and ADRB2 Arg/Arg and ACE II. Patients having ACE ID and ADRB2 Arg/Arg and ACE II and DRD1 GG genotypes were associated with a significant increase in diastolic blood pressure and heart rate at Ti, respectively (p<0.01, p<0.001).

Conclusions: These results indicate that single nucleotide polymorphisms in DRD1, ADRB3 and ACE genes may have an impact on hemodynamic changes during laryngoscopy/intubation/extubation.

Key Words: Cardiovascular response, intubation, extubation, polymorphism

Genetik Varyantlar ve Laryngoskopİ/Entübasyon/Ekstübasyon Srasındaki Kan Basıncı ve Kan Akımı Değerlerinde Oluşturulan Farklılıklar

Amaç: İlaç yanıtlarında bireyler arasında genetik yapıya bağlı değişiklikler vardır. Çalışmamızda laringoskopı ve trakeal entübasyon/ekstübasyonun mekanik stimülasyonuyla oluşan geçici hipertansiyonun anjiotensin konvertin enzimi (ACE) I/D, beta2-adrenerjik reseptör (ADRB2) Arg16Gly ve dopamin D1 reseptör (DRD1)-48 G/A polimorfizmlerinin olası ilişki araştırıldığındı.

Gereç ve Yöntem: ASA 1 (American Society of Anesthesiologists), 100 hasta çalışmaya alındı. Kalp hızı ve kan basınçını, induksiyon öncesi (T0), induksiyon sonrası (T1), laringoskopı/entübasyon sırasında (Ti) ve sonraki 1., 2., 3., 4. ve 5. dakikalarda (T1-1-T1-5), ekstübasyon öncesi (T2) ve sonrası 1., 2., 3., 4. ve 5. dakikalarda (T2-1- T2-5) kaydedildi. (ACE) I/D, (ADRB2) Arg16Gly ve (DRD1)-48 G/A polimorfizmleri polimeraz zincir tepkimesi ve restriksiyon parçacık uzuınlık polimorfizmi yöntemi ile araştırıldı. Veriler, faktöriyel düzeninde tek bir doz kullanılarakML comparatif analizleri ile değerlendirildi.

Bulgular: Laringoskopı, entübasyon ve ekstübasyon sırasında kan basıncı, kalp hızı ve hız-basınç ürünü (RPP) değerlerinde artış, 5 dk. içinde başlangıç değerlerine dönüş göründü. T1, ile karşılaştırıldığında T3,deki artış tüm değerler için anlamındı (p<0.01, p<0.001). T1,de, sistolik ve diastolik kan basınçları ve DRD1 -48 G/G genotipi arasında ve kat泼 hız ile ADRB2 Arg/Arg ve ACE II arasında ilişkilendirildi. ACE I/D ve ADRB2 Arg/Arg ve ACE II ve DRD1 G/G genotipi hastalar, T1,de kalp hızı ve diastolik kan basıncında anlamlı artışa (p<0.01, p<0.001). Sonuç: Bulgularımız ıflıota DRD1, ADRB2 ve ACE genlerindeki SNP’lerin, laringoskopı/entübasyon/ekstübasyon sırasında hemodinamik değişiklikler üzerinde etkili olabileceğini düşündüldü.

Anahtar Sözcükler: Kardiyovasküller yanıt, entübasyon, ekstübasyon, polimorfizm
Introduction

Hemodynamic instability, including hypertension, tachycardia and dysrhythmias, is mediated by noxious stimulus that provokes sympathoadrenal responses induced by the mechanical stimulation of laryngoscopic tracheal intubation, and it is important for anesthetists to recognize, diagnose and treat the condition (1,2). Therefore, the regulation and control of arterial pressure is essential. However, the precise molecular mechanism of the transitory hypertension induced by the mechanical stimulation remains to be elucidated.

The great individual hemodynamic response variety observed following mechanic stimulation is an emerging concept. For the management of transitory hypertension and tachycardia, several opioids, local anesthetics, β-blockers, calcium channel blockers, and α2-adrenoceptor agonists, etc. have been used with varying success rates (3-8).

Today’s molecular knowledge points out that gene polymorphisms will have a profound effect on human health by playing a role not only in the explanation of an individual’s disease susceptibility but in drug efficacy as well. Therefore, several single nucleotide polymorphisms (SNP) in the genes encoding the proteins that have a role in regulation of hypertension may have a major impact on our understanding of transitory hypertension, which is now accepted as a complex disease in which genetic and environmental components contribute to the clinical phenotype.

The renin-angiotensin system is involved in blood pressure regulation. The insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene is known to be associated with variation in plasma and cellular ACE concentrations. Furthermore, changes in arterial function have been suggested to be associated with the DD genotype (9).

The β2-adrenergic receptor (ADRB2) is expressed in a variety of tissue and vascular smooth muscle cells. It has a vital role in blood pressure (BP) regulation by mediating cardiac chronotropy, renal sodium excretion and vascular tone. Detailed molecular analysis reveals ADRB2 as a quantitative trait locus for BP (10). Among the several SNPs defined, ADRB2 Arg16Gly polymorphism has been reported to be associated with down-regulation of the receptor. Gly 16 allele is associated with an increased propensity for down-regulation of the receptor. It may cause an impaired vasodilation in peripheral arteries in response to peripheral vasoconstriction (11).

Dopamine has been shown to influence renal sodium excretion through a direct interaction with the dopamine receptor (DR). The dopamine D1 receptor (DRD1) has been localized to the proximal tubules and is known to increase sodium excretion by inhibiting Na-H exchange and Na,K-ATPase activity. Defective renal dopamine production and/or DR function have been reported in essential hypertension (EH) (12).

The present study was therefore conducted to examine the possible association of ACE I/D, ADRB2 Arg16Gly and DRD1 - 48 G/A gene polymorphisms and transient hypertension induced by the mechanical stimulation of laryngoscopic tracheal intubation in Turkish patients.

Materials and Methods

Patients

One hundred (53 male, 47 female) ASA (American Society of Anesthesiologists) class I patients aged 20-50 (mean ± SEM: 35.59 ± 0.99) years were enrolled in this study. Patient selection was made carefully in order to make a standard group since the polymorphisms could be related to other clinical parameters. Thus, all patients were subjected to elective septorhinoplasty or lumbar laminectomy as the surgical procedure. None of the patients had a family or personal history of hypertension or systemic diseases including cardiovascular pathologies. Difficult intubations were excluded from the study. All patients tolerated the extubation well; there was no respiratory distress or need for reintubation. Obese patients were also excluded from the study. Duration of the operations was not longer than three hours.

Operational Procedure

Patients were scheduled for elective surgery requiring general anesthesia with orotracheal intubation, and received diazepam 5 mg and famotidine 40 mg orally the night before surgery and midazolam 0.1 mg kg⁻¹ orally 60 min prior to anesthesia induction. Electrocardiogram (ECG), non-invasive BP, arterial oxygen saturation by pulse oximetry, and end-tidal CO₂ concentration by capnography were monitored. Baseline heart rate and BP values were recorded after 30 min rest in the operation room (T₀) and at following time points: after induction
(T_i), during laryngoscopy/intubation (T_i) and 1, 2, 3, 4 and 5 minutes thereafter (T_i-1-T_i-5), before extubation (T_e) and 1, 2, 3, 4 and 5 minutes afterwards (T_e-1-T_e-5).

Standard anesthetic induction procedure was used in all patients with 5-7 mg kg\(^{-1}\) thiopental, 0.1 mg kg\(^{-1}\) vecuronium and 1 µg kg\(^{-1}\) fentanyl. Patients were intubated by the same anesthesiologist on first attempt using a standard Macintosh laryngoscope blade and conventional tracheal tubes with high volume, low-pressure cuffs. Anesthesia was maintained with 40% O\(_2\), 60% N\(_2\)O and 0.5-2% isoflurane.

DNA Preparation

The ethics committee of Başkent University Faculty of Medicine in Ankara approved the study protocol. Genomic DNA was prepared from leukocyte pellets by sodium dodecyl sulphate (SDS) lysis, ammonium acetate extraction and ethanol precipitation (13) and used as a template for the polymerase chain reaction (PCR) with the primers as previously described (12,14,15).

Genotyping

**ACE I/D genotyping:** After amplification, PCR products were separated by electrophoresis on 2% agarose gel and identified by ethidium bromide staining.

The product was a 190 bp and 490 bp for D and I alleles, respectively. Thus, each DNA sample revealed one of the three possible patterns at the end of electrophoresis: a 190 bp band (genotype DD), a 490 bp band (genotype II) or both the 190 and 490 bp bands (genotype ID) (15) (Figure 1a).

**Beta2 Adrenergic Receptor (ADRB2) Arg16Gly polymorphism:** A 201 bp PCR product was cut with BsrDI for ADRB\(_2\) Arg16Gly polymorphism. The three fragments (131+56+14 bp) showed the presence of Arg16 allele. If the PCR product was cut into four fragments as 108+56+23+14 bp, it revealed the Gly16 allele (14) (Figure 1b).

**Dopamine D1 Receptor (DRD1)–48G/A polymorphism:** In order to analyze the DRD1–48G/A polymorphism, the Ddel digested PCR products were separated by 12% PAGE. The ethidium bromide stained gel showed three bands (217+146+42 bp) for A allele. In the presence of G allele, the PCR product (405 bp) was cut into two fragments of 146 and 259 bp (12) (Figure 1c).

Statistical Analyses

Since systolic BP, diastolic BP, heart rate and rate pressure product (RPP) were measured repeatedly at different time points for all sample members,
Kolmogorov Smirnov normality test and two factor Repeated Measures Factorial Analysis of Variance were used for data analyses. The differences between the genotypes were tested by using a two factor repeated measures ANOVA with physiological parameters as the repeated measure. Then, multiple comparisons were carried out according to Duncan and Bonferroni post-hoc methods. P value less than 0.05 was considered significant. The power of the statistical test was greater than 0.80 for all comparisons. All results are expressed as mean ± SEM (X ± s). Kolmogorov Smirnov test was performed by MINITAB 13.0 and other methods were performed by SPSS 11.5.

Results

During laryngoscopy, intubation and extubation, BP, heart rate and RPP increased. These values returned to baseline, in some cases to below baseline values, within five minutes following the procedures.

Blood pressure: During laryngoscopy and intubation (T<sub>i</sub> period), systolic and diastolic BPs increased, with a statistically significant difference (p<0.001) between the values recorded at T<sub>0</sub> and T<sub>i</sub> (Figure 2). Systolic and diastolic BPs reached maximum values at T<sub>i</sub> and started to decrease at 1 minute after intubation and gradually dropped to values below baseline.

During the extubation period, systolic BP at the 1<sup>st</sup> and 2<sup>nd</sup> minutes after extubation (T<sub>e-1</sub> and T<sub>e-2</sub>) were significantly higher than the pre-extubation values (p<0.05) (Figure 2). After the 2<sup>nd</sup> minute, pre-extubation values were reached.

Heart rate: During laryngoscopy and intubation period (T<sub>i</sub>), heart rate reached maximum values. Following the first minute after intubation, heart rate returned to the initial values. At T<sub>e</sub>, the heart rate values were significantly higher than the initial values (p<0.001). No statistically significant changes were observed in the heart rate during the extubation period (Figure 3).

Rate pressure product (RPP): RPP was calculated by multiplying systolic BP and heart rate. RPP increased significantly at T<sub>i</sub> (p<0.001) when compared with T<sub>0</sub> and decreased afterwards, falling below baseline values. Before extubation, RPP was close to baseline value, increasing slightly at the 1<sup>st</sup> and 2<sup>nd</sup> minutes after extubation and returning to pre-extubation values afterwards (Figure 4).

Genotypes: Genotype distribution of the patients and the association with hemodynamic parameters are shown in Tables 1 and 2.

ACE and ADRB2 genotypes did not seem to have a statistically significant effect on systolic BP as DRR<sub>1</sub> genotypes. The patients with -48GG genotype had the highest difference with respect to systolic BP (p<0.001).
When diastolic BP was taken into account, DRD1 -48GG genotype was found to be associated with a statistically significant increase in diastolic BP in Ti ($p<0.001$). ACE and ADRB2 genotypes did not seem to have such an effect on diastolic BPs.

When the heart rate was considered, DRD1 genotypes did not demonstrate an association with heart rate, as observed with ACE and ADRB2 genes ($p<0.05$). In patients with ACE II genotype, heart rate increased at T1 when compared to T0 and Ti ($p<0.001$). ADRB2 Arg/Arg genotype revealed similar results ($p<0.001$) (Table 1).

Patients with ACE ID and ADRB2 Arg/Arg genotype combination had a statistically significant increase in diastolic BP when compared at T0 and Ti ($p<0.001$). Combination of ACE II and DRD1 -48G genotypes seemed to cause a statistically significant increase in heart rate at T1 when compared to T0 and Ti ($p<0.001$).

**Discussion**

Laryngoscopy and tracheal intubation are the most invasive stimuli in the practice of general anesthesia, the influence of which on heart rate and BP has long been recognized. The cardiovascular response to this invasive stimulus arises from sympathoadrenal reflexes evoked by the stimulation of laryngeal and tracheal tissues. Although the hemodynamic response to this mechanical stimulation is short-lived, it may cause serious problems in patients with cardiovascular or cerebral disease. In this study, we determined ACE I/D, ADRB2 Arg16Gly and DRD1 –48G/A polymorphisms, which are thought to be related to the transient hypertension induced by the mechanical stimulation of laryngoscopy and tracheal intubation/extubation, and evaluated the potential influence of genotype variations on the intensity of the response.

All hemodynamic parameters increased significantly during intubation (Ti) and extubation (Te-1 and Te-2).

### Table 1. Association between genotypes and hemodynamic parameters at time points (Ti) with significant increases.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype (n)</th>
<th>Systolic blood pressure</th>
<th>Diastolic blood pressure</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T0 ± s0</td>
<td>T1 ± s1</td>
<td>T0 ± s0</td>
</tr>
<tr>
<td>ACE</td>
<td>DD (63)</td>
<td>129.20 ± 2.75</td>
<td>144.61 ± 3.98**</td>
<td>75.76 ± 1.76</td>
</tr>
<tr>
<td></td>
<td>ID (26)</td>
<td>133.92 ± 4.46</td>
<td>156.04 ± 6.47**</td>
<td>81.67 ± 2.22</td>
</tr>
<tr>
<td></td>
<td>II (11)</td>
<td>131.10 ± 5.77</td>
<td>149.50 ± 8.36**</td>
<td>78.15 ± 3.69</td>
</tr>
<tr>
<td>ADRB2</td>
<td>16 Arg/Arg (44)</td>
<td>133.92 ± 4.02</td>
<td>152.13 ± 5.83**</td>
<td>78.89 ± 2.57</td>
</tr>
<tr>
<td></td>
<td>16 Arg/Gly (28)</td>
<td>129.86 ± 3.91</td>
<td>144.50 ± 5.68**</td>
<td>75.83 ± 2.50</td>
</tr>
<tr>
<td></td>
<td>16 Gly/Gly (28)</td>
<td>129.32 ± 4.12</td>
<td>151.04 ± 5.98**</td>
<td>79.74 ± 2.64</td>
</tr>
<tr>
<td>DRD1</td>
<td>-48 AA (25)</td>
<td>127.42 ± 3.60</td>
<td>148.65 ± 5.20**</td>
<td>77.99 ± 2.30</td>
</tr>
<tr>
<td></td>
<td>-48 AG (61)</td>
<td>131.53 ± 3.30</td>
<td>142.26 ± 4.78**</td>
<td>80.34 ± 2.11</td>
</tr>
<tr>
<td></td>
<td>-48 GG (14)</td>
<td>134.14 ± 5.07</td>
<td>158.76 ± 7.35**</td>
<td>76.24 ± 3.24</td>
</tr>
</tbody>
</table>

** : $p<0.01$

*** : $p<0.001$
returning to baseline, and in some cases to below baseline values, within five minutes following the procedures. Similar results have been reported previously (16,17). Shribman et al. (17) reported a significant increase in systolic BP after laryngoscopy or laryngoscopy and intubation. They also reported a significant increase in heart rate in both laryngoscopy and laryngoscopy and intubation groups. In their study, Kim et al. (16) reported a significant increase in mean BP, heart rate and RPP values. Lasocki et al. (9) reported the influence of I/D polymorphism of the ACE gene on vasomotor properties. The angiotensin I-converting enzyme (ACE) is a dipeptidyl carboxypeptidase that is expressed in many tissues, including vascular endothelial cells. ACE has a role in BP regulation and electrolyte balance by hydrolyzing angiotensin I into angiotensin II—a potent vasopressor, and hence it is also able to inactivate bradykinin, a potent vasodilator. A common I/D polymorphism of the human ACE gene is associated with quantitative differences in circulating ACE activities, which is a well-known, albeit modest, risk factor for vascular disease. There are significant differences in the allele frequencies of the relevant polymorphism. This observed heterogeneity is normal because of the gene pool of our population. However, the two reports are inconsistent for Anatolia, which can reflect the genotype frequencies for a healthy Turkish population (18,19).

Among our patients, the frequencies of the DD, ID and II genotypes were 63, 26 and 11%, respectively, and no association was found with the increases in BP. However, the heart rate increased significantly in patients with ACE II genotype. Lasocki et al. (9) reported an increase in BP with increase in blood flow indicating a lesser decrease in vascular resistance. Our results did not support this finding. BP is a complex trait determined by an array of interlocking homeostatic systems with feedbacks that maintain homeostasis in the face of widely varying environmental factors.

Stimulation of B adrenergic receptors results in an increase in BP, heart rate, catecholamine and renin release (16). Genetic variations of the B2 – adrenoceptor gene may thus affect these parameters during laryngoscopy and intubation. In the present study, the prevalences of the Arg/Arg, Arg/Gly and Gly/Gly genotypes were 44, 28 and 28%, respectively, and the genotypes did not have a statistically significant association with systolic and diastolic BPs. However, increase in heart rate in patients with ADRB2 Arg/Arg genotype was highest compared to the other genotypes. Kim et al. (16) did not find a relationship between ADRB2 Arg16Gly polymorphism and mean BP and RPP. Talke et al. (20) recently studied the effect of ADRB2 Arg/Gly polymorphism in healthy volunteers and did not show any effect of the polymorphism on peripheral

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype (n)</th>
<th>Systolic blood pressure</th>
<th>Diastolic blood pressure</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T_0 ± s_0</td>
<td>T_1 ± s_1</td>
<td>T_0 ± s_0</td>
</tr>
<tr>
<td>DD (63)</td>
<td>126.92 ± 2.75</td>
<td>136.55 ± 2.81</td>
<td>77.08 ± 2.34</td>
<td>79.46 ± 2.27</td>
</tr>
<tr>
<td>ID (26)</td>
<td>129.20 ± 4.47</td>
<td>141.30 ± 4.56</td>
<td>80.04 ± 3.81</td>
<td>85.43 ± 3.68</td>
</tr>
<tr>
<td>II (11)</td>
<td>123.65 ± 5.77</td>
<td>127.60 ± 5.90</td>
<td>79.25 ± 4.92</td>
<td>70.65 ± 4.76</td>
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<tr>
<td>ACE</td>
<td>16 Arg/Arg (44)</td>
<td>137.30 ± 4.11</td>
<td>79.22 ± 3.32</td>
<td>83.88 ± 3.25</td>
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<td>16 Arg/Gly (28)</td>
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<td>82.54 ± 3.23</td>
<td>87.73 ± 3.16</td>
</tr>
<tr>
<td></td>
<td>16 Gly/Gly (28)</td>
<td>133.20 ± 4.22</td>
<td>76.77 ± 3.40</td>
<td>89.16 ± 3.33</td>
</tr>
<tr>
<td>ADRB2</td>
<td>48 AA (25)</td>
<td>135.11 ± 3.67</td>
<td>84.71 ± 2.96</td>
<td>87.93 ± 2.90</td>
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<tr>
<td></td>
<td>48 AG (61)</td>
<td>135.92 ± 3.37</td>
<td>79.60 ± 2.72</td>
<td>93.54 ± 3.24</td>
</tr>
<tr>
<td></td>
<td>48 GG (14)</td>
<td>136.86 ± 5.18</td>
<td>74.48 ± 4.18</td>
<td>83.08 ± 2.66</td>
</tr>
</tbody>
</table>

** : p<0.01
vasoconstriction. Our findings were to some extent in accordance with their reports; however, we found an association between ADRB2 Arg/Arg genotype and heart rate increase.

The distributions of DRD1 -48 AA, AG and GG genotypes were 25, 61 and 14%, respectively, in our patients. The patients with -48 GG genotype had the highest difference with respect to both systolic and diastolic BPs, whereas no association was found with heart rate. Sato et al. (12) reported an association between DRD1 polymorphism and essential hypertension. Krushkal et al. (21) also suggested the involvement of the DRD1 gene in the regulation of arterial pressure. Our findings support these reports.

With regard to the combination of genotypes, ACE ID and ADRB2 Arg/Arg genotypes were found to be associated with increased diastolic BP and ACE II and DRD1 -48 GG genotypes with increased heart rate. D allele of the ACE gene has been reported to be in association with an increased vascular reactivity (22). Also, the $\beta_2$ adrenoceptor gene has been reported to be associated with the pressor response to laryngoscopy and tracheal intubation (8).

In conclusion, we demonstrated significant association of the ACE I/D, ADRB2 Arg16Gly and DRD1 48G/A gene polymorphisms with hemodynamic response induced by the mechanical stimulation of laryngoscopy and tracheal intubation/extubation. Nevertheless, it may be possible that other known, or as yet unknown, SNPs within these genes or other genes could still be important in the pathogenesis of hemodynamic response. With this in mind, further research is needed to explore the exact molecular mechanism of hemodynamic response. In doing so, areas for therapeutic intervention using personalized medicine might be successfully implemented and be helpful for anesthesiologists in the management of the transient hypertension induced by the mechanical stimulation.

References


