The Effect of Degenerated Neuron Density of Petrosal Ganglion on the Development of Blood Pressure Variabilities after Subarachnoid Hemorrhage in a Rabbit Model: An Experimental Study

Tavşan Modelinde Subaraknoid Kanama Sonrası Gelişen Kan Basıncı Değişikliklerine Petrosal Gangliondaki Dejenere Nöron Dansitesinin Etkisi: Deneysel Çalışma

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ABSTRACT

AIM: The aim of this study was to determine the relationship between ischemic neurodegeneration, of the petrosal ganglion of the glossopharyngeal nerve, and BP fluctuations, after subarachnoid hemorrhage (SAH).

MATERIAL and METHODS: Twenty-four rabbits had their blood pressure and heart rhythms studied daily over 20 days. Then, the histopathology of the petrosal ganglion was examined in all animals. Normal and apoptotic neuron density of the petrosal ganglion and blood pressure values were compared statistically.

RESULTS: Mean total volume of the petrosal ganglia was calculated as $0.9 \pm 0.34/mm^3$. BP level of control group was $96.1 \pm 2.1 mmHg$; $116.5 \pm 4mmHg$ of mild hypertension (HT) group and $128.1 \pm 3.6mmHg$ in the severe HT group. When the groups were compared to each other they were significantly different. The level of normal-apoptotic neuron in control group was $11240 \pm 802/mm^3$-$40 \pm 6.3/mm^3$; $9730 \pm 148.7/mm^3$-$1560 \pm 256.2/mm^3$ in the mild HT group and $6870 \pm 378.8/mm^3$-$4240 \pm 628.2/mm^3$ in the severe HT group. When the groups were compared to each other there was significantly difference.

CONCLUSION: Blood pressure variability observed in this study may be explained by ischemic neurodegeneration of petrosal ganglia caused by SAH. The results of this study suggest that petrosal ganglion ischemia has potential implications for the development of hypertension. These findings suggest that new treatment strategies should be considered for the treatment of SAH.

KEYWORDS: Glossopharyngeal nerve, Petrosal ganglion, Neuron density, Blood pressure, Subarachnoid hemorrhage

ÖZ

AMAÇ: Bu çalışmanın amacı, subaraknoid kanama (SAK) sonrası, glossofaringeal sinir petrozal ganglion iskemik nörodejenerasyonu ve KB dalgalanmaları arasındaki ilişkiyi belirlemektir.

YÖNTEM ve GEREÇLER: Yirmi dört tavşanın kan basıncı ve kalp ritmi 20 gün boyunca günlük olarak takip edildi. Daha sonra, tüm hayvanların petrozal ganglionu histopatolojik olarak incelenmiştir. Petrozal gangliondaki normal ve apoptotik nöron yoğunluğu ile kan basıncı değerleri istatistiksel olarak karşılaştırıldı.

BULGULAR: Petrozal ganglionun ortalama toplam hacmi $0,9 \pm 0,34 / mm^3$ olarak hesaplandı. Kontrol grubunda KB düzeyi $96,1 \pm 2,1 mmHg$ idi. Hafif hipertansiyon (HT) grubunun $116,5 \pm 4mmHg$ ve ağır HT grubunda $128,1 \pm 3,6 mmHg$ idi, gruplar birbirleriyle karşılaştırıldığında gruplar arasında anlamlı fark bulundu. Kontrol grubunda normal-apoptotik nöron düzeyi $11.240 \pm 802/mm^3$-$40 \pm 6,3 / mm^3$ idi hafif HT grubunda $9730 \pm 148,7/mm^3$ - $1560 \pm 256,2/mm^3$ ve ağır HT grubunda $6870 \pm 378,8/mm^3$-$4240 \pm 628,2/mm^3$. Gruplar birbirleriyle karşılaştırıldığında anlamlı fark vardı.

SONUÇ: Bu çalışmada, kan basıncı değişkenliklerinin SAK'ın petrozal ganglionda iskemik nörodejenerasyona neden olması ile açıklanabileceği görülmüştür. Bu çalışmanın sonuçları, petrozal ganglion iskemisinin hipertansiyon gelişimine potansiyel etkileri olduğunu göstermiştir. Bu bulgular yeni tedavi stratejilerinin SAK tedavisi için düşünülmesi gerektiğini göstermektedir.

ANAHTAR SÖZCÜKLER: Glossofaringeal sinir, Petrozal ganglion, Nöron dansitesi, Kan basıncı, Subaraknoid kanama

INTRODUCTION

Blood pressure (BP), the result of neural innervation from several systems, contributes to the autonomic control of blood flow in the body. When arterial pressure rises, increased activity in the baroreceptor afferents activates medullary pathways that reduce arterial pressure by inhibition of sympathetic vasomotor activity, which reduces the heart rate by activation of parasympathetic cardiac vagal efferents. When arterial pressure falls, the reduced baroreceptor activation leads to increased sympathetic vasomotor activity and decreased vagal activity (35). Baroreceptor nerve endings that innervate the aortic arch and carotid sinus detect acute fluctuations in the arterial pressure. The nerve endings of the glossopharyngeal and vagal nerves detect acute fluctuations in arterial pressure (9). Nerve terminals of the baroreceptor reflexes are regulated by glossopharyngeal nerves (GPN). The pericaria of these visceral afferent neurons are located in the inferior sensory ganglia. Peripheral (17) or nuclear (22) GPN injuries may result in hypertensive crises.

SAH caused by an aneurysm rupture is a devastating condition that carries significant morbidity and mortality. Because of the delayed narrowing of large-capacity arteries of the brain, cerebral vasospasm is one of the leading causes of morbidity and death following SAH (5). The main cause of an abrupt fall in BP has been attributed to brain dysfunction (31). All neurons generate electrical impulses when they are exposed to ischemia (10). In this study, the neurons of the petrosal ganglia were quantified in normotensive and hypertensive rabbits to determine whether the number of neurons in the petrosal ganglia played a role in BP levels. According to results reported by Costa et al., (10) ischemic discharge of the petrosal ganglion may be responsible for hypotension at the onset of a subarachnoid hemorrhage. However, ischemic degeneration of petrosal ganglion neurons may cause hypertension due to the effects of the interruption of the parasympathetic pathway on BP. The relationship between ischemic neurodegeneration of the petrosal ganglion of the GPN and BP fluctuations was studied in a rabbit model of SAH.

MATERIAL and METHODS

This study was conducted on 24 hybrid rabbits. Experiments were carried according to the guidelines set by the ethical committee of our Institute. All animals were divided into three groups:

1. Control group (BP<100mmHg) (n=8)

2. SAH with mild hypertension (BP:100-120mmHg) (n=8)

3. SAH with severe hypertension (BP>120mmHg)(n=8)

All brains, brainstems, lungs, hearts and other organs were preserved in 10% formalin solution. The petrosal ganglions were obtained and examined from the normotensive and hypertensive animals.

A balanced, injectable anesthetic was used to reduce pain and mortality. After anesthesia was induced with isoflurane,

given by facemask; 0.2 mL/kg of the anesthetic combination: 150 mg/1.5 mL Ketamine HCL, 30 mg/1.5 mL Xylazine HCL, and 1 mL distilled water was subcutaneously injected before surgery. During the procedure, a dose of 0.1 mL/kg of the anesthetic combination was used when needed.

Eight animals (n=8) were used as controls. The remaining (n=16) rabbits were kept without food for six hours before surgery. Autologous blood (1 mL) was taken from the auricular arteries and injected, using a 22-gauge needle, into the cisterna magna of the animals in the SAH group over the course of one minute. The animals in the control group were not subjected to this procedure. All animals were monitored for BP, heart rhythm, respiration and blood gas saturation under anesthesia before the SAH, during surgery, and post procedure, five times a day. The BP values were invasively obtained from the femoral artery (Siemens SC-7000 ASA-model no: 5202994-Electromedical Group-USA). The animals were followed for 20 days without any medical treatment and then were sacrificed. The entire body of all animals was kept in 10% formalin solution after preparation for histological examination. BP values and quantification of the neuronal density of the petrosal ganglion of the GPN were examined histopathologically.

In order to estimate the neuron density of the petrosal ganglion, all of the GPN with their ganglion were extracted bilaterally below the jugular foramen. Then, they were longitudinally embedded in paraffin blocks for histopathological examination of the petrosal ganglion. The samples were stained with Tunnel dye. The Stereology and Cavalieri method was used to evaluate the density of the neurons in the petrosal ganglions. Cellular angulation, nuclear shrinkage, cytoplasm condensation, and height loss were considered signs of injury of the petrosal ganglions using H&E staining. In addition, light-blue cells were considered Tunnel negative and dark-brown cells Tunnel positive.

To estimate the density of intact and degenerated neurons, for each ganglion, the stereological method was used (15, 18, 29). This method is useful for quantification and is easily performed; it does not rely on assumptions about a particular shape, size or orientation, or unaffected overprotection and truncation. Two consecutive sections obtained from tissue samples were used as references and mounted on slides. The reference and look-up sections were reversed in order to double the number of dissected pairs without obtaining new sections (Figure 1A-B). If the same cell was seen in reference and mounted section, only the cell in reference section was added to calculation to avoid the recalculation. Volumetric calculation of the cell value was counted through the ratio of the cell number per unit volume. The ratio of the cell number in reference section to whole sections was calculated. This ratio was multiplied with whole volume (whole thickness X surface are). Neuron number in whole ganglion was estimated through this formula.

The mean numerical density of the normal and degenerated neurons in the petrosal ganglion (Nv/Gv) per mm^3 was determined using the formula Nv/Gv=ΣQ-N/ΣAxd where

SQ-N was the total number of counted neurons found only in the reference sections, d was the section thickness, and A was the area of the counting frame. The most effective way of estimating ΣA, for the dissection set, was via ΣA=ΣP.a. ΣP was the total number of points in the counting set frame and a was a constant area associated a set point. The physical dissector method of stereology was used to evaluate the number of neurons in the petrosal ganglion and GPN axons.

The Cavalieri volume estimation method was used to obtain the total volume of tissues and cells for each specimen. The total number of neurons was calculated by multiplying the volume (mm^3) by the numerical density of the neurons for each petrosal ganglion (Figure 1A,B).

The differences between the density of degenerated neurons of the petrosal ganglion of the GPN and BP values were compared statistically.

Statistical Methods

Statistical calculations were performed with SPSS 17 ™ Inc. (Statistical Package for Social Sciences) for Windows. In addition to standard descriptive statistical calculations (mean and standard deviation), BP levels and histological results were analyzed using linear regression test on regression of the variables, one-way ANOVA test on comparison of the parametric variables and Wilcoxon Signed Rank test in comparisons of the non-parametric variables. Values of $p < 0.05$ were considered as statistically significant.

RESULTS

The results of the evaluations of the heart, respirations, blood gases and BP of the normal animals are shown in Figure 2A. A summary of the animals with a SAH is shown in Figure 2B. At the onset of the SAH, sinus bradycardia and hypotension were observed in all animals (<150 beats/min). Later, premature
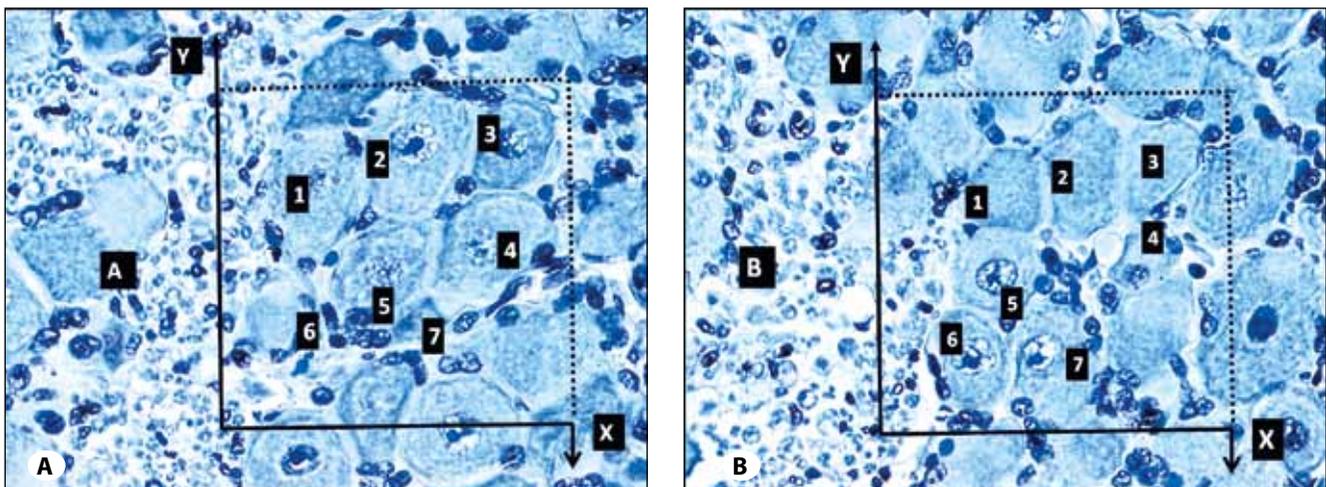

Figure 1A-B: Two parallel adjacent thin sections separated by a distance of 5 micrometer. Upper and right lines of unbiased counting frames represent the inclusion lines and the lower and left lines including the extensions are exclusion lines. The nucleoli marked with '1,2,3,4' are dissector particles on A section as it disappeared section B. The nucleoli marked with '5,6,7' are not dissector particles on A section as it disappeared section B. The neurons of outside of the square are not included (Tunnel Stain, x100, LM).
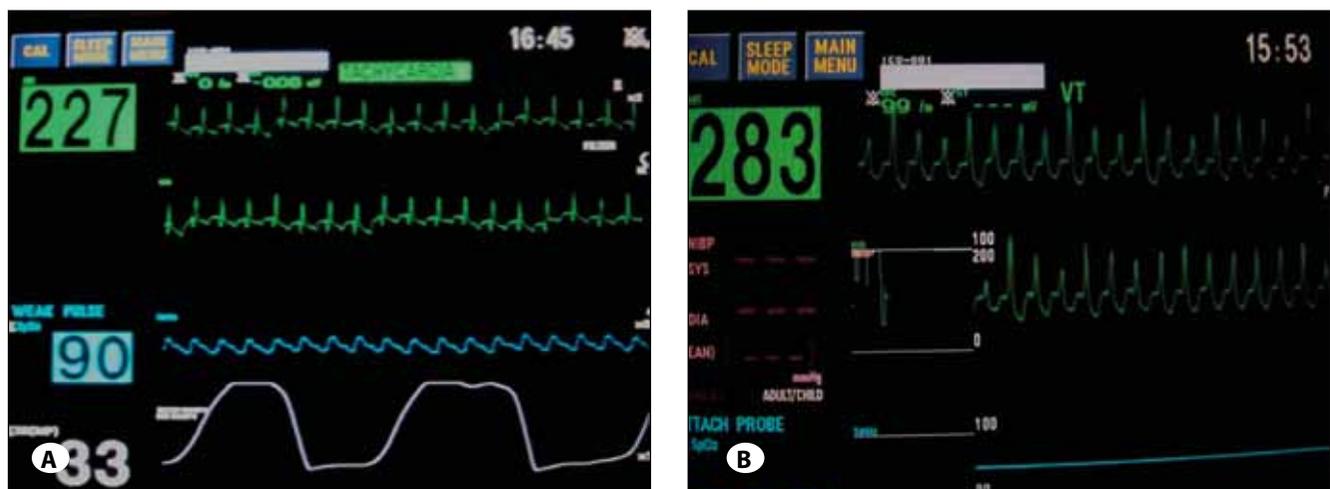

Figure 2: A) Electrocardiogram of normal rabbit. **B)** Electrocardiogram of a rabbit with subarachnoid hemorrhage.

supraventricular/ventricular contractions, polygeminy and atrioventricular heart block were identified on the ECGs in the animals with petrosal ganglion degeneration. Seven days after the SAH, extranodal tachycardia episodes were detected in animals with severe petrosal ganglion neuron degeneration (greater than 60%). At the time of death, tachycardia decreased and cardiac arrhythmias and idioventricular heart rhythms were observed. With regard to the cardiac arrhythmias, the frequency of supraventricular/ventricular premature contractions in the acute phase was significantly higher than during the chronic phase.
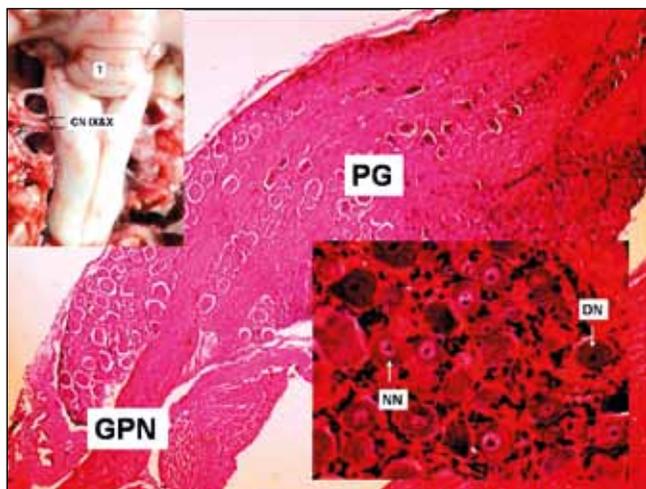

Figure 3: Macroscopical (Left upper corner - Glossopharyngeal (CN IX) and vagal nerves (CN X) are seen between the brainstem and jugular foramen (T-Tonsilla cerebelli) and Histopathological appearance of glossopharyngeal nerve (GPN) and petrosal ganglion (PG) are seen (LM, H&E, x40). At the right bottom, a magnified form of PG neurons are seen (LM, H&E, x100; NN-Normal neuron; DN-Degenerated neuron).

In this study, the mean total volume of the petrosal ganglia was calculated as $0.9\pm0.34mm^3$. Figure 3 shows the macroscopic (left upper corner) and microscopic appearances (background) of normal petrosal ganglion. Figure 4 shows the histopathological appearance of a bloody petrosal ganglion from an animal with SAH. The histological cytoarchitecture of a petrosal ganglion and neuron are illustrated in Figure 1A and B dyed with Tunnel stain.

The values of BP, level of normal neuron and apoptotic neurons respect to control, mild HT and severe HT was summarized in Figure-5. BP level of control group was 96.1±2.1mmHg; 116.5±4mmHg of mild HT group and 128.1±3.6 mmHg in severe HT group. When the groups were compared to each other they were significantly different from each other (p=0.012) (Figure 5).

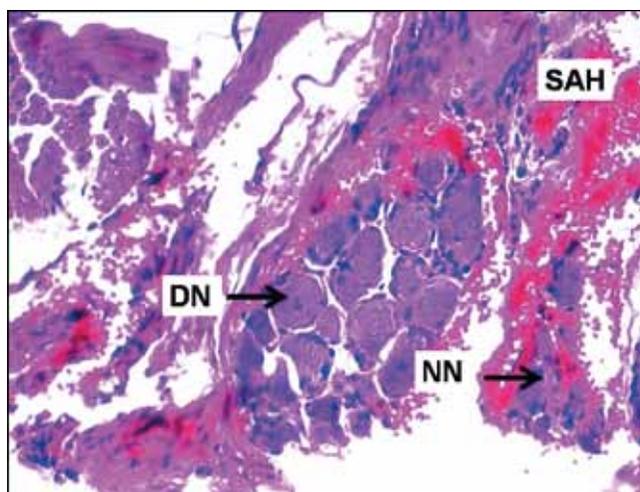**Figure 4:** Microscopical appearance of a bloody petrosal ganglion after subarachnoid hemorrhage (LM,H&E,x100).
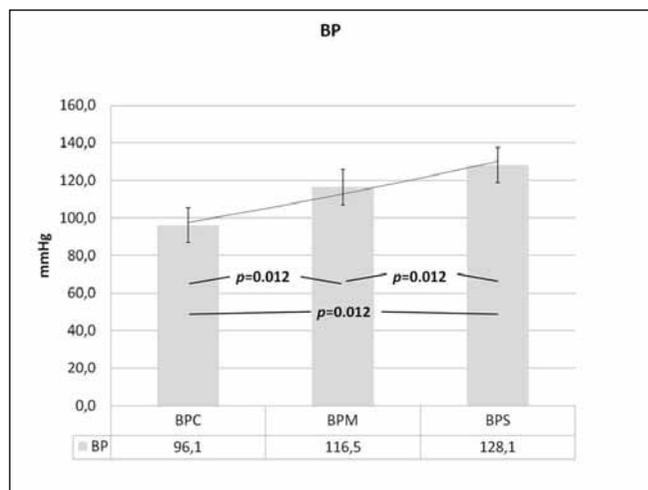

Figure 5: Mean blood pressure (BP) levels of the groups and the correlation of the groups to each other. When the control (BPC), mild hypertension (BPM) and severe hypertension (BPS) groups were correlated p values were 0,012.
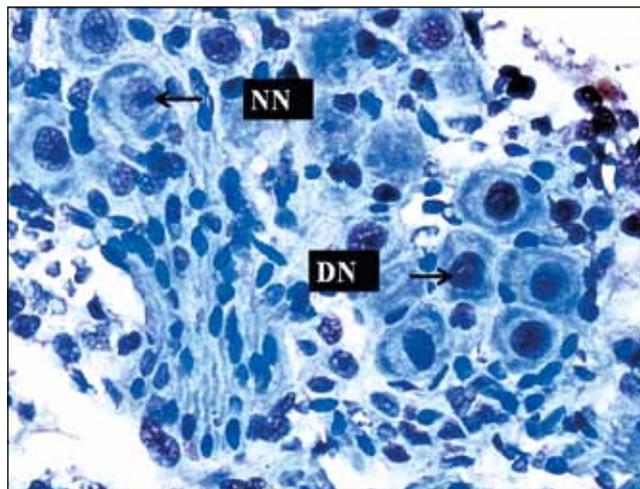

Figure 6: Some apoptotic neurons (DN) are detected among the normal neurons (NN) in the petrosal ganglions of hypertension developed animals with subarachnoid hemorrhage (H&E, x100, LM).

The level of normal neuron in control group was 11240±802/ mm³; 9730±148.7/mm³ in mild HT group and 6870±378.8/ mm³ in severe HT group. When the groups were compared to

each other they were significantly different from each other (p=0.012) (Figure 6-7). The level of normal neuron in control group was 40±6.3/mm³; 1560±256.2/mm³ in mild HT group and 4240±628.2/mm³ in the severe HT group. When the groups were compared to each other they were significantly different from each other (p=0.012) (Figure 6-7). The ratio of apoptotic/normal neuron was 0.3% in control, 16% in mild HT and 61.7% in severe HT group.
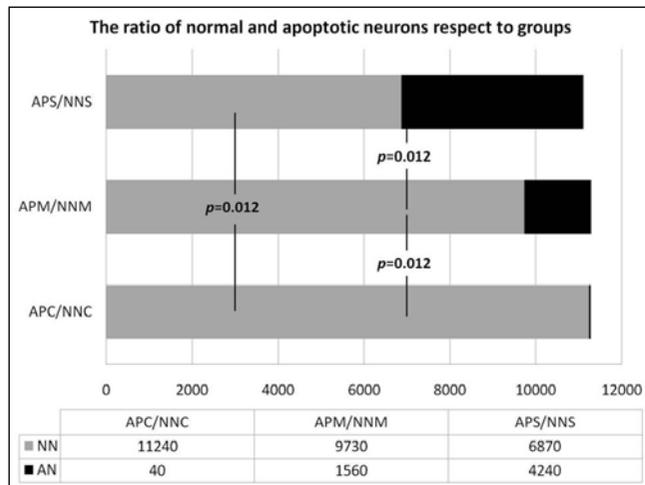
The level of normal neurons and apoptotic neurons showed significant regression to BP (p<0.001) (Figure 8). Beta level was 0.008 in normal neuron level and - 0.008 in apoptotic neuron levels. In summary, SAH resulted in neuronal apoptosis of the petrosal ganglion. The results suggest that the apoptotic neuron density played a major role in the development of hypertension associated with SAH.

Figure 7: Normal neuron (NN) and apoptotic neuron (AP) values of control (APC/NNC), mild hypertension (APM/NNM) and severe hypertension (APS/NNS) groups and their ratio was illustrated. When the NN values and AN values of control, mild hypertension and severe hypertension groups were correlated p values were 0,012.

DISCUSSION

The effects of the sympathetic nervous system on Blood Pressure (BP) regulation is well established (13). There is also evidence of involvement of the parasympathetic nervous system on hypertension (4); the neural regulation of BP is mainly effected by the interplay of the sympathetic and vagal neural pathways. These pathways compose the neural circuitry contained in the brain stem and in the basal forebrain.
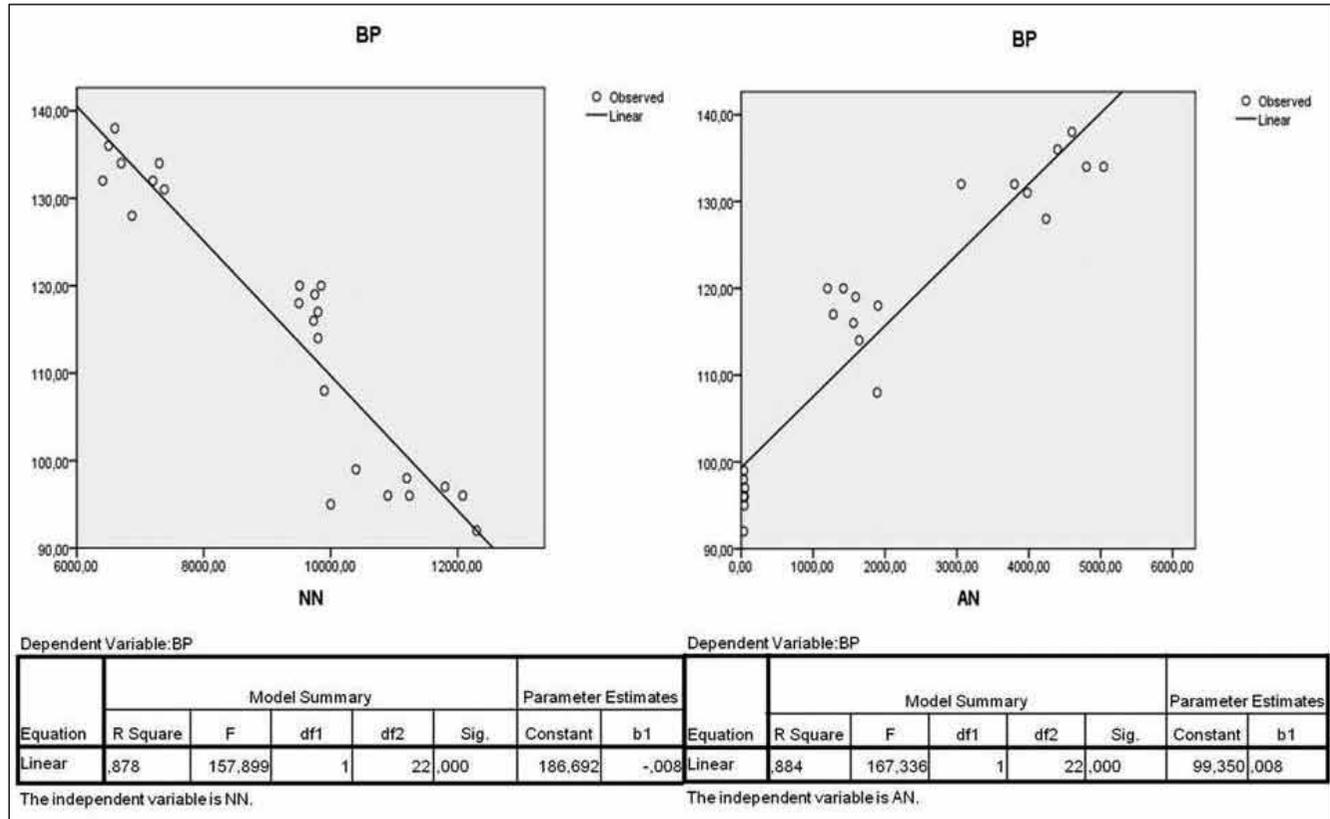

Figure 8: Linear regression of NN value (left) and AN value (right) to BP was illustrated in the in the graphics. Beta values were given in the tables under the graphics. The level of normal neurons and apoptotic neurons showed significant regression to BP (p<0.001). Beta level was 0,008 in normal neuron level and -0,008 in apoptotic neuron levels.

The nerve endings of the GPN and vagal nerve are sensitive to acute fluctuations in arterial pressure; this is because nerve terminals of the GPN regulate baroreceptor reflexes. Aydin et al. (1) showed that the neuron density of petrosal ganglion has important effects on the determination of BP.

In this study, rabbits were evaluated for the association between apoptotic neurons in the petrosal ganglion 20 days after a SAH; one BP measure was obtained days before the SAH. The results of this study suggest that the BP variability observed after the SAH was caused by changes associated with ganglion degeneration. Goadsby (16) showed that electrical stimulation of the greater petrosal nerve, facial nerve or pterygopalatine ganglion increased cortical or cerebral blood flow in anesthetized rats and dogs; the response was mediated by non-cholinergic factors (30). However, D'Alecy et al. (11) reported that cholinergic mechanisms played a role. Parasympathetic nuclei in the brainstem send preganglionic fibers through the geniculate ganglion, such as the greater petrosal nerve, to the pterygopalatine ganglion; the nerve cells of this ganglion give rise to the postganglionic fibers of the nasal and lacrimal glands and possibly the cerebral vasculature (33).

According to Faraci and Heistad, large cerebral arteries significantly contribute to the regulation of cerebral vascular resistance and blood flow in the cat and monkey (14). Electrical stimulation of the sphenopalatine ganglion or electrical microstimulation of the basal forebrain increases cortical blood flow in anesthetized rats (25). The autonomic nervous system maintains cardiovascular homeostasis with regard to cardiac performance through the opposing effects of the parasympathetic and sympathetic pathways and by the predominant sympathetic control of the vasculature.

The main cause of an abrupt fall in BP associated with a SAH has been attributed to brain dysfunction (31). All neurons generate electrical impulses when exposed to ischemia (10). Despite intensive research efforts, BP instability and vasospasm after SAH remains incompletely understood from both the pathogenic and therapeutic perspectives. According to the results of Costa et al. (10) and this study, ischemic discharges of the petrosal ganglion may be responsible for hypotension at the onset of SAH. However, ischemic degeneration of the petrosal ganglion may also generate hypertension due to the interruption of the parasympathetic pathway and the resulting effects on BP; the findings of this study support this mechanism.

Importance of the Present Study

Gustatoreceptor, baroreceptor and chemoreceptor parasympathetic fibers of the GPN have an anatomical and physiological connection in the petrosal ganglion, salivatory nucleus and nucleus tractus solitarius of the brain stem (8). Some parasympathetic fibers of the GPN enter the intralingual ganglia at the posterior portion of the tongue and their postganglionic fibers innervate the lingual glands and taste buds (8). Aydin et al. (2) showed that the

antihypertensive effects of nifedipine are related to the taste bud receptor stimulating effects of the GPN. The sensitive fibers of the GPN innervating the carotid body and carotid sinus, together with the taste buds, are localized within the petrosal and nodose ganglia (21). The neuronal information, conveyed to the petrosal and nodose ganglia, is sent to the inferior salivary nucleus and nucleus tractus solitarius. The information from the GPN that reaches these two nuclei is integrated into the intra- and inter-nuclear neural networks. The taste information is transmitted to baroregulatory and chemoregulatory centers within the brain stem. Taste, pressure and chemosensitive impulses reaching the brain stem affect each other. The brain stem regulates the BP and pH according to the taste information received by the GPN (6, 19, 24, 34).

The normal systolic BP of a rabbit is 94±5 mmHg (1). Although the BP usually decreases during the first hours of SAH, increased BP values are recorded later (26). These findings are explained by the results of the present study. Degeneration of the inferior sensory ganglia of the GPN was observed in the animals with hypertension. A sudden rise in arterial pressure stimulates the primary afferent baroreceptor nerves entering the brain stem as part of the GPN and vagal nerve. The paired nuclei of the solitary tract are the principal central termination sites of the baroreceptor afferents. Interruption of the baroreceptor afferents to the solitary nucleus or damage to afferent baroreceptor neurons may result in fulminant hypertension (20).

Visceral sensory neurons of the GPN are located in the petrosal ganglia. Peripheral axotomy of the petrosal ganglion may result in a decrease of BP (20). GPN injury and nuclear GPN lesions may result in hypertensive episodes (22). Injury of the petrosal ganglion may also lead to experimental hypertension (9). In addition, percutaneous radiofrequency administration to the petrous ganglion, at the jugular foramen, for the treatment of glossopharyngeal neuralgia, can result in bradycardia and hypotension (28).

Cerebral vasospasm causes neuronal and astrocytic apoptosis with SAH (27). Aydin et al. (3) showed that SAH causes ischemic insults to the vagal nerve ganglia. Therefore, SAH may also cause ischemic neurodegeneration in the petrosal ganglia and result in hypertension by decreasing the number of neurons in these ganglia (1). The regression of normal neuron and apoptotic neuron level to the BP was shown in this study.

Focal cerebral ischemia caused apoptotic cell death in the petrosal ganglia. Apoptosis is an active process of cell death that can be identified by Tunnel staining (12). Immunostaining studies have reported cell swelling, cytoplasm vacuolization, loss of membrane integrity, nuclear fermentation without nuclear condensation, as the indices of necrotic cell morphology (32). In the present study, vasospasm of the arteries supplying the petrosal ganglion might have caused the neuronal apoptosis.

CONCLUSION

Several recent investigations have reported BP variation associated with a SAH. Aneurysmal SAH can be associated with acute global and regional decreases in the cerebral perfusion. Furthermore, cerebral vasospasm may lead to development of delayed ischemic deficits (23). Systolic BP level and range might be important for the management of patients with a SAH and may influence patient outcome (7). Therefore, it is important to determine the mechanisms associated with the regulation of BP after SAH.

The results of this study suggest that ischemic discharges of the petrosal ganglion may be responsible for hypotension at the onset of SAH. However, the ischemic degeneration of the petrosal ganglion causes hypertension due to the interruption of the parasympathetic pathway and its effects on BP. Increase in the glosopharyngeal input may result in hypotension and decrease in the glossopharyngeal nerve input might result in hypertension.

In conclusion, neurodegeneration of the petrosal ganglion may play an important role in the increase of BP. If afferent baroreceptor nerve or solitary nucleus injuries can cause fulminant hypertension, a reduced number of neurons in the petrosal or nodose ganglia might play an important role in the etiology of hypertension. The results of this study showed inverse proportion between the normal number of neurons in the petrosal ganglion and BP values. High doses of anti-oxidant treatment or stimulation of GPN can help by the treatment of hypertension in SAH or preservation of normal BP levels. Additional studies are needed to further elucidate the role of neuronal density in the etiology of hypertension.

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