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Research Article

Effect of intra-arterial heparin flushing (IAHF) to prestin and vascular endothelial growth factor (VEGF) level in hearing loss patients

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Abstract

Background: According to the World Health Organization (WHO), hearing loss is the fourth largest disability globally, affecting an estimated 466 million people in 2018. In Indonesia, the prevalence of hearing loss was estimated at 16.8% in 2016. Intra-Arterial Heparin Flushing (IAHF) is an endovascular technique that uses heparin to promote reperfusion and increases Vascular Endothelial Growth Factor (VEGF) expression. VEGF is a polypeptide angiogenesis factor present in the nervous system, which functions as a neurotrophic and neuroprotective. Meanwhile, prestin is a protein of outer ear hair cells that shows early signs of hearing loss through increased levels in cases of ear hair cell damage.

Objective: This study aims to evaluate the effect of IAHF on prestin and VEGF levels in hearing-impaired patients.

Methods: The design is experimental with Pre-Post Test One-Group Only. A total of 22 patients with hearing loss were measured for prestin and VEGF before and 4 hours after the IAHF procedure.

Results: The results of the Wilcoxon test showed no significant differences in prestin level (p=0.658) and VEGF level (p=0.291) before and after the IAHF procedure. The mean showed an insignificant decrease in prestin level before and after the IAHF procedure with values of 1,185+1,229 pg/mL and 1,096+1,183 pg/mL, respectively. However, the VEGF level insignificantly increased before and after the procedure with values of 484.83+274.6 pg/mL and 498.79+257.7 pg/mL, respectively.

Conclusion: There were no significant differences in prestin and VEGF levels before and after the IAHF procedure. However, there was a decrease in the prestin level and an increase in the VEGF level.

Keywords

intra-arterial heparin flushing, Prestin, VEGF, hearing loss

Introduction

According to the World Health Organization (WHO), hearing disorder is the fourth largest disability globally, affecting 466 million people in 2018. In Indonesia, the prevalence of hearing disorder was estimated at 16.8% in 2016. Despite the high prevalence rate, most patients are unaware of their conditions, leading to inadequate treatment. However, only one-third of people with hearing disorder are aware of their problem (Djaali et al. 2018; WHO 2018; Michels et al. 2019).

Intra-Arterial Heparin Flushing (IAHF) is an endovascular technique that uses heparin to achieve reperfusion. Previous investigations have shown that IAHF, a modified technique of Digital Subtraction Angiography (DSA), can lead to clinical improvement for stroke patients in Gatot Soebroto Army Central Hospital. This condition can also increase Cerebral Blood Flow (CBF) in chronic ischemic stroke (Putranto et al. 2016a; Ratmono et al. 2016).

Heparin has been shown to release myeloperoxidase enzyme binding with endothelium, thereby increasing the bioavailability of Nitric Oxide (NO). The increase in NO can lead to high VEGF expression, which has been linked to improvement in hearing in animals with Noise-Induced Hearing Loss (NIHL) (Jozkowicz et al. 2001; Baldus et al. 2006; London and Gurgel 2014). VEGF is a factor of polypeptide angiogenic growth that is also present in the neuro system. It plays a crucial in the angiogenesis process and defense mechanism that can help supply oxygen and nutrition to ischemic brain tissue. VEGF also works directly with neurons to produce neurotrophic and neuroprotective effects. Some studies have also mentioned that VEGF induces axonal growth and neuron survival (Zhang et al. 2000; Jin et al. 2002; Yang et al. 2010) and plays a role in neurogenesis by inducing the release of neurotrophic factor (Thau-Zuchman et al. 2010; Nowacka and Obuchowicz 2013). An investigation in poultry showed the effect of VEGF to regenerate hair cells and supporting cells in the ear (Wan et al. 2020).

Prestin is a protein of outer ear hair cells that can be used as a biomarker to detect hearing loss. An increase in prestin levels indicates damage to the outer ear hair cell, which is an early sign of hearing loss (Tovi et al. 2019; Sun et al. 2020). Damaged hair cells released prestin serum into circulation. Studies have found increased prestin in Idiopathic Sudden Sensorineural Hearing Loss (ISSHL), which is identified as a marker of auditory hair cell integrity. Prestin is also recommended as prognostic to evaluate the effectiveness of treatment and improvement of hearing threshold in ISSHL (Naples et al. 2017; Tovi et al. 2019; Sun et al. 2020; Gomaa et al. 2021).

IAHF procedure has the potential to recover macro and micro circulation, and heparin also possesses an anti-oxidant attribute. Therefore, this study aims to determine the effect of IAHF on prestin and VEGF levels in patients with hearing loss. The results are expected to help the sensorineural hearing loss caused by decreased vascularization and endothelium dysfunction.

Material and method

Method

This experimental study was carried out using a Pre-Post Test One Group Only in the Cerebrovascular Center Gatot Soebroto Army Central Hospital Jakarta. The prestin and VEGF level was measured before and 4 hours after IAHF without any control group in Kramat Prodia Laboratory Clinic in Central Jakarta, from November 2021 to Mei 2022. This study has received approval from Ethical Committee for Health Research Universitas Sumatera Utara in the approval letter number 615/KEP/USU/2021. Based on the sample size calculation, the minimum sample was 21 participants. Consecutive sampling was used to gather all subjects, which was a type of non-probability sampling technique. All subjects were expected to fulfill the inclusion and exclusion criteria. The inclusion criteria were patients with sensorineural and mixed hearing loss, not pregnant, and those who agreed to sign informed consent, have prestin and VEGF level measurement. The exclusion criteria were patients with conductive type hearing loss, those with contrast allergy or heparin, blood clotting disorder, and a history of severe illness such as renal failure, cardiac decompensation, and malignancy.

Material

For IAHF, the fluoroscope Artis Q Zen produced by Siemens and a Terumo guide wire was used to reach the designated location. The materials used also included Heparin 5,000 IU by Inviclot, Normal Saline by Otsuka, Bethadine's Povidon Iodin 10%, Alcohol 70%, lidocaine 2%, and Visipaque contrast produced by General Electric Healthcare in the United States. Human Prestin Enzyme–Linked Immunosorbent Assay (ELISA) Kit used was produced by MyBioSource in San Diego, California, United States, and VEGF ELISA Kit used was Quantikinie ELISA produced by Bio-Techne, Minneapolis, Minnesota, United States.

Study procedure

Before the IAHF procedure, patients with conductive type hearing loss or normal hearing were excluded through audiometry examination. To ensure safety, the potential side effects such as blood clotting were fully explained to the patients and their families. Subsequently, the patients were included in the IAHF procedure after they agreed to the possibility of side effects.

The IAHF procedure was carried out under sterile conditions. The equipment used included a catheter, introducer sheath, guide wire, lidocaine 2% in a 10 ml syringe, and mixed heparin 5,000 IU into 500 cc normal saline. Local anaesthesia was administered through lidocaine intracutaneous and subcutaneously. Access from the femoral artery was made with venous catheter 18G and the guide wire was inserted through fluoroscopy to ensure sure proper positioning. After removing the venous catheter, the introducer sheath was inserted, while its cap and guide wire was taken off simultaneously. Subsequently, the sheath was flushed with heparin to ensure there were no air bubbles. The angiography catheter was inserted using a guide wire until it reached the carotid artery. The angiography was performed using contrast Visipaque, while the carotid and vertebral arteries were flushed with 200 ml and 100 ml of heparin, respectively, at a pressure of 2 ml/s. The catheter was removed, followed by the sheath, and any bleeding was stopped by applying pressure with a gauze or using an angio seal.

The procedure for prestin and VEGF by ELISA method was carried out using prepared serum, then measured by spectrophotometer. Subsequently, prestin and VEGF levels were measured before and 4 hours after the IAHF procedure.

Data analysis

Data analysis in this study was carried out using SPSS (Statistical Program for Social Sciences) version 26. The normality of the data was determined by using the Shapiro-Wilk test, as the sample was expected to be <50. When the data were normally distributed, a paired t-test was used for hypothesis testing, otherwise, the Wilcoxon test was applied. Based on the statistical results, a significant difference between groups was indicated by a p-value less than 0.05, while a p-value higher than 0.05 indicated no difference.

Data availability

Data will be made available on request.

Results and discussion

Sample characteristics

This study involved 220 patients who passed through an audiometry examination. A total of 83 patients were in the normal hearing category, while 41 patients who had conductive-type hearing loss were excluded. After providing additional education and explanation about the procedure, 74 patients denied to participate and only 22 agreed to proceed. The differences among the patients were characterized and explained in Table 1.

Table 1. Characteristics of patients.

Category	N	%
Gender		
Male	15	68.2
Female	7	31.8
Age		
<60	7	31.8
≥60	15	68.2
Occupation		
Civil Servant	1	4.5
Entrepreneur	13	59.1
Housewife	6	27.3
Unemployed	2	9.1
Comorbidities		
1 Comorbidity	6	27.2
>1 Comorbidity	16	72.7
Type of hearing loss		
Sensorineural	17	77.3
Mixed	5	22.7
Degree of hearing loss		
Mild	10	45.5
Moderate	9	40.9
Moderate-severe	1	4.5
Severe	2	9.1
Very Severe	0	0

Out of the 22 patients, 15 (68.2%) were male, 15 (68.2%) were >60 years old, and the largest occupation group was entrepreneur, with 13 (59.1%). Furthermore, 16 (72.7%) of the patients have >1 comorbidity from various diseases such as hypertension, diabetic mellitus, dyslipidemia, stroke, and even COVID-19. The patient's characteristics based on their degree of hearing loss were also categorized as presented in Table 2.

Table 2. Patient characteristics based on degree of hearing loss.

Characteristics	Degree of hearing loss				
	Mild	Moderate	Moderate-severe	Severe	Very severe
Gender					
Male	8 (36.4%)	6 (27.3%)	0	1 (4.5%)	0
Female	2 (9.1%)	3 (13.6%)	1 (4.5%)	1 (4.5%)	0
Age					
<60	3 (13.6%)	4 (18.2%)	0	0	0
≥60	7 (31.8%)	5 (22.7%)	1 (4.5%)	2 (9.1%)	0
Occupation					
Civil servant	1 (4.5%)	0	0	0	0
Entrepreneur	6 (27.3%)	6 (27.3%)	1 (4.5%)	0	0
Housewife	2 (9.1%)	3 (13.6%)	0	1 (4.5%)	0
Unemployed	1 (4.5%)	0	0	1 (4.5%)	0
Comorbidities					
1 Comorbidity	4 (18.2%)	2 (9.1%)	0	0	0
>1 Comorbidity	6 (27.3%)	7 (31.8%)	1 (4.5%)	2 (9.1%)	0

Prestin level analysis

Based on the normality data test in Table 3, the prestin level was not normally distributed, hence, the statistical analysis was continued using the Wilcoxon test.

The Wilcoxon test showed that there was no significant difference in prestin level before and after the

IAHF procedure, with p-value >0.05 (p=0.658; 95% CI), as shown in Table 4. Based on the mean, a decrease in prestin level before and after the IAHF procedure was obtained, with values of 1,185+1,229 pg/mL and 1,096+1,183 pg/mL, respectively. The graph of prestin level before and after the IAHF procedure can be seen in Fig. 1.

Table 3. Analysis of the prestin level normality test.

	Prestin level (pg/mL)		
	Mean±SD	Median (Min–Max)	*P*
Pre IAHF	1,185±1.229	519.52 (227–3.963)	0.000[a]
4 hours Post IAHF	1,096±1.183	513.89 (239–3.069)	0.000[a]

[a]Shapiro-Wilk test.

Table 4. Analysis of prestin level before and after procedure.

	Prestin Level (pg/mL)		
	Mean±SD	Median (Min–Max)	*P*
Pre IAHF	1,185±1,229	519.52 (227–3,963)	0.548[b]
4 hours Post IAHF	1,096±1,183	513.89 (239–3,069)	

[b]Wilcoxon test.

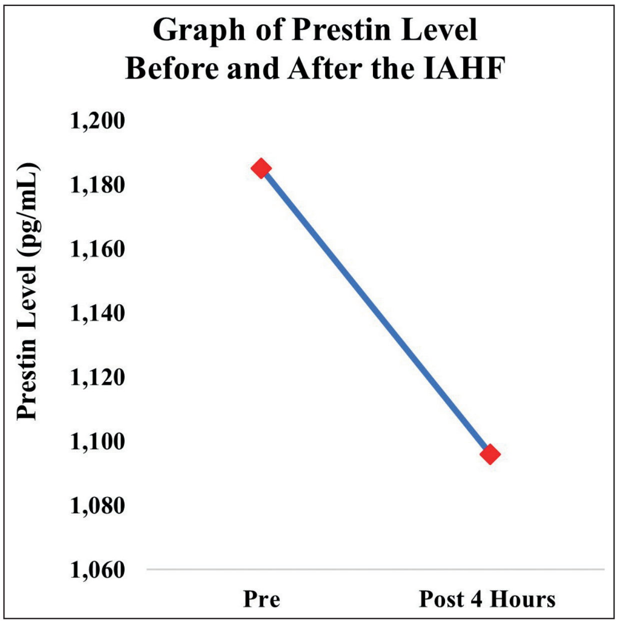

Figure 1. Graph of prestin level before and after procedure.

VEGF level analysis

Based on the normality data test in Table 5, the VEGF level was not normally distributed, hence, statistical analysis was carried out using the Wilcoxon test.

The Wilcoxon test showed no significant differences in VEGF level before and after the IAHF procedure, with a p-value of >0.05 (p=0.291; 95% CI), as shown in Table 6. However, the mean showed an increase in VEGF level before and after the IAHF procedure, with values of 484.83+274.6 pg/mL and 498.79+257.7 pg/mL, respectively. The graph of VEGF level before and after the IAHF procedure can be seen in Fig. 2.

Table 5. Analysis of the VEGF level normality test.

	VEGF Level (pg/mL)		
	Mean±SD	Median (Min–Max)	*P*
Pre IAHF	484.83±274.6	485.35 (76–1,337)	0.023[a]
4 hours Post IAHF	498.79±257.7	500.45 (86–1,220)	0.223[a]

[a]Shapiro-Wilk test.

Table 6. Analysis of VEGF level before and after procedure.

	VEGF Level (pg/mL)		
	Mean±SD	Median (Min-Max)	*P*
Pre IAHF	484.83±274.6	485.35 (76–1,337)	0.291[b]
4 hours Post IAHF	498.79±257.7	500.45 (86–1,220)	

[b]Wilcoxon test.

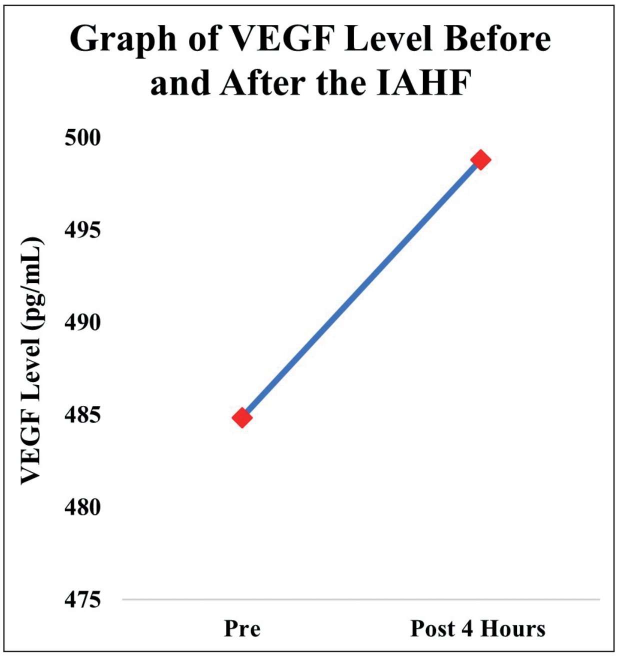

Figure 2. Graph of VEGF level before and after procedure.

Discussion

IAHF was performed in several cases such as ischemic stroke, thrombotic cerebral vein, autism, and stenosis renal artery. Meanwhile, the IAHF procedure has been associated with an increase in CBF in the infarct area, as demonstrated by the Magnetic Resonance Imaging (MRI) before and after IAHF. This improvement in CBF resulted from the clot lysis and vasodilation that enhance macro and micro blood circulation and vasodilatation, rather than angiogenesis (Slevin et al. 2006; Angels Font et al. 2010; Perrey 2013; Putranto et al. 2016b, 2019; Ratmono et al. 2016; Pramono et al. 2019; Setiawan et al. 2019).

Heparin is an anti-coagulant that can be administered directly through IAHF. Based on previous investigations, heparin has several pharmacologic attributes such as anti-inflammation, anti-thrombotic, pro-fibrinolysis, anti-aggregative, anti-proliferation, anti-oxidant, and anti-ischemic (Shute et al. 2018; Pramono et al. 2019; Putra et al. 2019). The anti-oxidant effect involved the myeloperoxidase enzyme, produced by leukocytes to decrease

NO levels by using it as the substrate to form free radicals. Heparin released myeloperoxidase enzyme binding with endothelium to increase NO bioavailability (Baldus et al. 2006).

Hair cell loss can occur happen through apoptotic or necrotic processes caused by cellular stress and ROS (Reactive Oxygen Species) (Peng and Jou 2010; Böttger and Schacht 2013; Dinh et al. 2015). Several reports have shown that the decrease in vascularization and endothelium dysfunction are pathogenic mechanisms for otologic disorders such as NIHL, presbycusis, and endolymphatic hydrops (Picciotti et al. 2004).

Prestin is a protein from outer ear hair cells that serves as a biomarker for assessing hearing function. Prestin concentrations increase in the presence of damaged outer ear hair cells. (C. Sun et al. 2020). It was also reported that VEGF in hearing improve in animals with noise-induced hearing loss on day-1 and day-7 after induction (London and Gurgel 2014).

Chadha et al. (2021) reported that more than 42% of people aged over 60 years experience hearing loss, and its prevalence increases with age (Chadha et al. 2021). In this study, a higher incidence of hearing loss was observed in patients aged >60 years old compared to those <60 years old, which was also consistent with the report of Shim et al. (2019). This indicated that an increase in age correlated with ear hair cell degeneration, supporting cell, as well as stria vascularis, and also induced nerve fiber decreased cochlear nerve. The central auditory nerve system also changed due to age increment in the auditory brain stem and auditory cortex (Fillit et al. 2016).

Study conducted by Gupta and friends showed that hearing loss prevalence was 2 times higher in males compared to females (Gupta et al. 2021), while others reported 1.1–1.43 times higher (Bansal et al. 2016). Similarly, this study obtained a hearing loss prevalence, with a comparison of 2.14:1 between males and females. This condition occurred because males were more probable to be exposed to noise in their workplace or recreational place. There was also a higher proportion of male smokers, which can lead to hearing loss because smoking has been linked to hearing loss. (Siegelaub et al. 1974). However, some studies reported that there is no correlation between gender and hearing loss in the non-industrial environment (Goycoolea et al. 1986; Helfer 2001).

There are several risk factors for hearing loss, namely inflammation process, systemic and genetic disease, as well as oxidative stress. Hearing loss had also been correlated with diabetes, cardiovascular such as increased blood pressure, and dyslipidemia (Sun et al. 2015).

Morbidity and mortality have also been correlated with hearing loss, primarily due to pathological mechanism of oxidative stress, vasoconstriction, and decreased blood flow that causes cochlear damage (Ohinata et al. 1994; Lee et al. 2001; Bamiou 2015; Oh et al. 2015; Rim et al. 2021).

In this study, patients with more than one comorbidity were more common compared to those with a single comorbidity. The comorbidities that were found included hypertension, diabetic mellitus, dyslipidemia, stroke, and COVID-19. This was similar to other investigations, where the incidence of hearing loss was in patients with metabolic syndrome (Aghazadeh-Attari et al. 2017; Shim et al. 2019).

This study showed that patients with sensorineural hearing loss were more compared to mixed type. Liu et al. discovered that the sensorineural type was the most common, followed by mixed, and conductive types. They also reported that most of the hearing loss degrees were mild and moderate, as confirmed by the dominant population in this study (Liu et al. 2011).

Prestin is produced by the cochlea and can be present in body circulation because it is small size enough to pass through the blood barrier. Prestin can be detected using ELISA in normal people as the result of outer ear hair changes (Parham and Dyhrfjeld-Johnsen 2016; Liba et al. 2017; Dogan et al. 2018; Parham et al. 2019; Tovi et al. 2019; Parker et al. 2022).

In this study, the prestin levels before and after the IAHF procedure was not significantly different but there was an 89 pg/mL mean difference. Based on previous investigations, prestin level was higher in ISSHL patients compared to those with non-hearing loss. However, there was no significant association between prestin levels before the IAHF procedure and hearing threshold improvement. (Sun et al. 2020). Hana et al. discovered that in 300 NIHL patients, there was a significantly decreased prestin level before and after therapy, with values of 169+88.4 pg/mL and 114+99.2 pg/mL, and a mean difference of 55 pg/mL (Hana and Bawi 2018).

The lack of insignificant results in this study can be due to factors outside the scope of outer ear hair cells, such as spiral ganglion dysfunction (Frisina et al.). Furthermore, prestin only showed outer ear hair cell condition and the IAHF procedure focused on oxidative stress treatment.

VEGF has a protective effect on neuron cells from hypoxia and ROS increment (Clinkard et al. 2013). London Gurgel (2014) also found that VEGF is beneficial as a therapy for sensorineural hearing loss (London and Gurgel 2014). In this study, VEGF measurement was carried out before and after the IAHF procedure. The results showed no significant difference between the two stages but the mean level was increased. The use of heparin as an agent in IAHF had been proven to increase NO bioavailability (Baldus et al. 2006) and induce VEGF expression (Jozkowicz et al. 2001). Furthermore, based on the use of the human umbilical vein, heparin increased VEGF expression in the first 24 hours by increasing Hypoxia Inducible Factors-1 (HIF-1) and AP-1 (Fazil et al. 2021).

An increased VEGF level has been associated with treatment in animal models of NIHL (Picciotti et al. 2005; Picciotti et al. 2006). Another report showed that VEGF inhibitor induced hearing loss due to decreased local blood flow and microvascular thrombosis in the labyrinth artery (Cheng et al. 2019).

The various VEGF levels obtained in this study might be due to influences from hypoxia, differentiation cell process, growth factor, and oncogenic (Kolch et al. 1995). The results showed that patients with comorbidity such as metabolic syndrome can have lower VEGF compared to normal people. According to Barylski et al. (2008), the more comorbidities a patient has, the lower the levels of initial VEGF (Barylski et al. 2008).

Conclusion

This study showed that most of the patients were over 60 years old and had mild to moderate hearing loss. The results indicated that the IAHF procedure in sensorineural and mixed-type hearing loss reduced prestin levels and increase VEGF, although the changes were not statistically significant. Therefore, future study should investigate the use of the IAHF procedure to treat hearing loss caused by endothelial dysfunction and vascular abnormalities in sensorineural and mixed-type cases.

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Declaration of interest

The authors declare no conflict of interest.

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