Unique Endovascular Repair
Spontaneous Aortocaval Fistula

Highlights

Case Report and Literature Review
Abdominal Compartment Syndrome

Discovering Thoughts, Inventing Future

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CONTENTS OF THE ISSUE

1. Spontaneous Aortocaval Fistula Associated with Ruptured Abdominal Aortic Aneurysm – Unique Endovascular Repair. 1-6
2. Tension Pneumoperitoneum and Abdominal Compartment Syndrome Rare Complication of Percutaneous Radiological Gastrostomy, Case Report and Literature Review. 7-12
3. The Role of 2D Speckle Tracking Echocardiography in Early Detection of Left Ventricular Dysfunction in Type II Diabetic Patients. 13-19
4. Cardiac Effects of (−)-Epigallocatechin on Isolated Rat Hearts. 21-24

v. Fellows
vi. Auxiliary Memberships
vii. Preferred Author Guidelines
viii. Index
Spontaneous Aortocaval Fistula Associated with Ruptured Abdominal Aortic Aneurysm—Unique Endovascular Repair

By Emilia M Krol MD, Dorothy A Sparks MD & Alan Dietzek MD FACS

Department of Surgery, Danbury Hospital

Abstract- Aortocaval fistula (ACF) is a rare complication associated with abdominal aortic aneurysm. Open repair of this entity is well described in the literature. There is paucity of data, however, on endovascular means for repair as well as mid- and long-term outcomes. We describe a case of a ruptured abdominal aortic aneurysm that presented with an aortocaval fistula that we managed with endovascular interventions on both the aorta and inferior vena cava. Following placement of an aortic stent graft, there was persistent flow through the ACF and a large Type I endoleak. Subsequent management included placement of second bifurcated stent graft in the inferior vena cava. No further endoleaks were encountered. To our knowledge, this represents unique management of a rare complication of aortic aneurysm rupture with complete endovascular exclusion of ACF.

Keywords: aortocaval fistula, endovascular repair, abdominal aortic aneurysm, inferior vena cava, type ii endoleak.

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Spontaneous Aortocaval Fistula Associated with Ruptured Abdominal Aortic Aneurysm – Unique Endovascular Repair

Emilia M Krol MD, Dorothy A Sparks MD & Alan Dietzek MD FACS

Abstract: Aortocaval fistula (ACF) is a rare complication associated with abdominal aortic aneurysm. Open repair of this entity is well described in the literature. There is paucity of data, however, on endovascular means for repair as well as mid- and long-term outcomes. We describe a case of a ruptured abdominal aortic aneurysm that presented with an aortocaval fistula that we managed with endovascular interventions on both the aorta and inferior vena cava. Following placement of an aortic stent graft, there was persistent flow through the ACF and a large Type I endoleak. Subsequent management included placement of second bifurcated stent graft in the inferior vena cava. No further endoleaks were encountered. To our knowledge, this represents unique management of a rare complication of aortic aneurysm rupture with complete endovascular exclusion of ACF.

Keywords: aortocaval fistula, endovascular repair, abdominal aortic aneurysm, inferior vena cava, type ii endoleak.

I. Introduction

Spontaneous aortocaval fistula (ACF) was described first by Syme in 18311, complicating a syphilitic aneurysm. It is a rare entity, associated with 1% - 4% of ruptured abdominal aortic aneurysms (AAA). The classic presentation of an ACF includes high–output cardiac failure, a palpable pulsatile abdominal mass and a machinery bruit. The presence of all of these symptoms occurs in only 20-50% of patients. Successful treatment of ACF by both open and endovascular methods has been reported, with mortality rates as high as 60% for the former and a paucity of data for the later3. There are no reported cases in which stent grafts were used to treat both the arterial and venous components of an ACF.

II. Case Presentation

A 61-year-old male was transferred to the Emergency Room from an outside hospital with a known ruptured AAA. Upon arrival, he was hypotensive complaining of severe abdominal pain radiating to the back with new- on- set paresthesia of his bilateral lower Extremities. The duration of symptoms was approximately two hours before evaluation in our institution. The patient was not in congestive heart failure. A CT Angiogram of the abdomen and pelvis was obtained and revealed a ruptured 7.5cm infrarenal AAA with contrast extravasations into the retro peritoneum. There was a significant contrast enhancement of the inferior vena cava (IVC) suggesting the presence of ACF (Fig 1). The aneurysm neck was highly angulated and approximately 12mm long. The patient was taken to our hybrid operating room (OR) for further treatment. Wire access was obtained through bilateral femoral artery cut-downs, and a Medtronic 26mm Talent main body device with AneuRxiliac limbs was placed with preservation of both hypo gastric arteries. Aortography revealed a large Type I end leak (EL) and persistent ACF (Fig 2, Fig 3). A large Palmaz stent was mal-deployed at the neck and was, therefore, moved proximally and fully deployed in the thoracic aorta. A second well deployed Palmaz stent failed to completely resolve the Type I EL as did placement of a proximal aortic cuff with intentional coverage of the left renal artery. The femoral veins were cannulated, and a large compliant balloon was inflated in the IVC to occlude the ACF with a resultant significant decrease in the Type I EL suggesting that closing the fistula by placing a stent graft within the inferior vena cava (IVC) at the bifurcation might resolve the large end leak. A sufficiently large stent graft was not available at the time so the patient was transferred to the ICU where he remained stable until he returned to the operating room on the second post-operative day. The ACF was excluded by a deployment of a Gore TAG device within the IVC followed by placement of bilateral iliac vein reversed Gore 16x20 limbs (Fig 4). Aortography demonstrated a small Type III end leak which was not treated at this point.

In the ensuing days, however, the patient’s hematocrit decreased and a repeat CTA showed the retroperitoneal pseudo aneurysm and aneurysm to have significantly increased in size, a recurrent ACF and both large Type I and III endoleaks and a right femoral DVT. The patient returned to the OR for possible endovascular or open aneurysm repair. During surgery, the left and right iliac stent grafts were relined with Gore excluder limbs, the Palmaz stent was once again

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repositioned into the thoracic aorta, and the proximal aortic stent graft was re-ballooned with a noncompliant balloon. All endoleaks were now resolved as was the ACF (Fig 5). An IVC filter was also placed. After this procedure, the patient’s post-operative course was uncomplicated and discharged home in good condition. At four year follow-up, the patient’s aneurysm remains excluded with continued sac shrinkage and no evidence of an ACF.

III. Discussion

The preoperative diagnosis of spontaneous ACF is crucial when planning AAA repair both in elective and emergency cases. Before endovascular techniques, ACF represented an unwelcome challenge to the vascular surgeon who attempted the open repair. The most common method used was over-sewing the fistula from within an opened aneurysm sack, but this is associated with significant blood loss and high mortality, even in elective cases 4, 6.

The first description of the use of endovascular stent grafts in the treatment of arteriovenous fistulas was described by Bougdhene et al. in an experimental study on a sheep model, where fistulas were created percutaneously7. This study was followed by a published case series by Juan C. Parodi8, in which he described the successful use of endovascular techniques for treatment in patients with arteriovenous fistulas as a result of a traumatic injury. The first use of endovascular exclusion for the treatment of ACF associated with AAA was described by Beveridge et al in 19989, and was followed by several similar reports 8-14. In all of these cases, implantation of an aortic stent graft proved adequate to resolve the ACF, and none of the patients were reported to require further treatment.

Cases in which placement of an aortic stent graft does not resolve an ACF remain a challenge for vascular surgeons. There have been three reports in the literature describing the use of Amplatzer plugs to occlude unresolved fistulas after aortic stent graft placement.15-17and one, describing the use of EmbikrilatBrauhistoacrylic gel18. In another report, a covered tubular stent graft, deployed in the IVC, was used to successfully treat a persistent ACF manifesting as a type II endoleak in a patient who had endovascular treatment of ruptured abdominal aortic aneurysm six months prior19.

Our patient had a preoperative diagnosis of a large aneurysm which had ruptured both into the IVC and into the retro peritoneum. Despite repair of the aortic rupture with a stent graft and additional procedures including a Palmaz stent and a proximal aortic cuff, there remained a large type IIa endoleak and ACF. Placement of a bifurcated aortic stent graft within the IVC to completely cover the ACF ultimately resolved the type IIa endoleak and the ACF. A possible theory for why this succeeded is that the presence of a large ACF behaved as a large, low pressure outflow sump making adequate sealing of the aortic stent graft impossible. With the closure of the venous portion of the ACF, the outflow transitioned to a high – resistance system, which eventually led to thrombosis of fistula and resolution of endoleak. Wang et al described their experience with three cases of endovascular repair of ACF with hostile aortic anatomy. In all cases only aortic repair was performed and two out of three patients suffered from early type 1 or 3 endoleak requiring reintervention20. Although there are anecdotal reports of successful endovascular treatment of aortic rupture with ACF by placement of an aortic stent graft alone, the size of the ACF is not described nor is the quality of the proximal aortic neck. In our case the ACF was large and the proximal aortic neck highly angulated and short, making it difficult to manage with only one stent-graft exclusion.

End luminal stent-graft repair of IVC and other vein injuries have been previously described, mainly for treatment of traumatic injuries21. No long-term results for these treatments have thus far been reported in the literature. Silveira et al described similar repair of ACF with coverage of the venous portion of the fistula with a stent graft cuff from the venous access22. Similarly, Elkassaby et al described two cases with a simultaneous deployment of aortic and IVC stent grafts to exclude ACF in two patients with ruptured AAA with good short-term results.23In our case, the midterm result has proved to be excellent and we will continue to monitor this patient for long-term treatment durability. Clearly, despite our good result, further reports of similar treatments are necessary before any relevant conclusions or recommendations can be made for this uncommon entity.

IV. Conclusion

Although aortocaval fistula, as a complication of a ruptured aortic aneurysm, remains a challenge in surgical management, endovascular treatment of such condition is feasible but may require both aortic and caval interventions for ultimate success.

References Références Referencias


Fig. 1: CTA demonstrating free rupture of a 7.5 infrarenal AAA with aortocaval fistula

Fig. 2: Successful deployment of stent graft within the AAA
Fig. 3: Persistent flow through ACF despite successfully deployed aortic stentgraft

Fig. 4: Successful deployment of stentgraft within the IVC, with persistent Type III endoleak
Fig. 5: Successful realignment of aortic and venous stent grafts, with resolution of ACF and end leaks
Tension Pneumoperitoneum and Abdominal Compartment Syndrome
Rare Complication of Percutaneous Radiological Gastrostomy, Case Report and Literature Review

By Abbas AR Mohamed, Turki Atia Al Quarshi & Abdulsalam A Bin Hafiz
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Abstract- Tension pneumoperitoneum (TP) is defined as the massive accumulation of air in the peritoneal cavity, which results in a sudden increase in intraabdominal pressure. Various iatrogenic procedures are responsible for this complication (1). We report a case of tension pneumoperitoneum after percutaneous radiological gastrostomy (PRG).

Keywords: percutaneous radiological gastrostomy, tension pneumoperitoneum, abdominal compartment syndrome.

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Tension pneumoperitoneum and Abdominal Compartment Syndrome Rare Complication of Percutaneous Radiological Gastrostomy, Case Report and Literature Review

Abbas AR Mohamed °, Turki Atia Al Quarshi ° & Abdulsalam A Bin Hafiz °

Abstract- Tension pneumoperitoneum (TP) is defined as the massive accumulation of air in the peritoneal cavity, which results in a sudden increase in intraabdominal pressure. Various iatrogenic procedures are responsible for this complication (1). We report a case of tension pneumoperitoneum after percutaneous radiological gastrostomy (PRG).

Keywords: percutaneous radiological gastrostomy, tension pneumoperitoneum, abdominal compartment syndrome.

I. Introduction

Tension pneumoperitoneum and abdominal compartment syndrome (ACS) are rare complications of percutaneous radiological gastrostomy. We report a case of tension pneumoperitoneum and compartment syndrome in a 14 years old girl as a complication of percutaneous radiological gastrostomy. We also review the literature for this rare complication.

II. Case Report

A 14 years old female known case of cerebral palsy, epilepsy, spinal deformities and fixed flexion deformities of upper and lower limbs who was in long-term nasogastric feeding was admitted electively to our hospital for insertion of percutaneous gastrostomy feeding tube. She had the procedure done under conscious sedation. Size 14 Fr feeding pigtail-retained catheter was inserted percutaneously into the stomach under fluoroscopic guidance. The patient tolerated the procedure well without immediate complications (figure 1) however, she failed to tolerate feeding through the gastrostomy tube and the tube had to be clamped. She also developed frequent vomiting and increasing abdominal distension over the forty-eight hours after the procedure.

Figure 1: Fluoroscopic view showing the feeding tube in the stomach

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She had abdominal x-rays which revealed suspicious of free intraperitoneal air (figure 2).

Figure 2: The abdominal X-Ray showing the feeding tube in the stomach and free air in the peritoneal cavity together with the vertebral column and limbs deformities.

She also had an urgent abdominal CT scan with gastrograffin contrast through the nasogastric tube which showed massive pneumoperitoneum with mild free fluids in the peritoneal cavity without evidence of leak (figure 3, 4).

Figure 3: The CT scan of the abdomen, showing marked pneumoperitoneum and the gastrostomy tube in the stomach without evident leak.
Shortly after the CT scan, the patient's condition deteriorated rapidly. She became confused with pulse rate over 130/minute and systolic blood pressure below 90 mm of Hg and she started to drop her oxygen saturation. Her abdomen became more distended, tympanic and rigid. She was rushed to the operation room for laparotomy. The abdomen was explored through an upper mid line incision. The patient had immediate response after entering the abdomen, her pulse rate started to come down and her blood pressure started to build up. The stomach was found not fixed to the anterior abdominal wall with about 5 cm of the tube between the stomach and the abdominal wall. The free intraperitoneal fluids were sucked out and testing for gastric leak by methylene blue injection through the nasogastric tube demonstrates leaking around the gastrotomy tube (photograph 1). The tube was secured to the stomach with double purse string sutures and the stomach was fixed to the anterior abdominal wall. The patient was kept ventilated for 24 hours and remained hemodynamically stable. Feeding through the gastrotomy tube was assumed on the second post-operative day and was well tolerated by the patient.

Photograph 1: Intraoperative photograph showing that the stomach was found not fixed to the anterior abdominal wall with about 5 cm of the tube between the stomach and the anterior abdominal wall with leakage of the methylene blue around the tube.
Tension Pneumoperitoneum and Abdominal Compartment Syndrome Rare Complication of Percutaneous Radiological Gastrostomy. Case Report and Literature Review

III. Discussion

Gastrostomy placement for nutritional support for patients with inadequate oral intake has been attempted using surgical, endoscopic, and, more recently, percutaneous radiologically guided methods. (2)

An open surgical gastrostomy was first described by Stamm in 1894 and was considered the standard for long-term enteral access and gastric decompression until percutaneous endoscopic gastrostomy (PEG) was introduced in 1980 by Gauderer et al. (3) Since its first description, percutaneous endoscopic gastrostomy (PEG) has become the method of choice for providing enteral access and nutritional support to patients who are unable to take oral feedings. (4) and it is almost totally replaced open feeding gastrostomy.

The percutaneous radiological gastrostomy (PRG), also known as "radiologically inserted gastrostomy," which uses fluoroscopic guidance, was first performed in 1981 by the Canadian surgeon Preshaw, (5,6) and was devised as an alternative to PEG in cases in which passing an endoscope is difficult or risky (6–8).

Another alternative to PEG tube is the laparoscopic approach described by Edelman et al (9, 10) in 1991. It has the same advantage of (PRG) in patients in whom endoscopy is not possible because of impassable obstructions from tumors in the pharyngoesophageal region or facial trauma. In addition, it offers the benefits of intra-abdominal inspection and therefore reduces the chances of injuries during the procedure (11).

Tension Pneumoperitoneum (TP) is defined as the massive accumulation of air in the peritoneal cavity, which results in a sudden increase in intraabdominal pressure. (1) it is a is a rare cause of abdominal compartment syndrome (ACS) with high intra-abdominal pressure caused by trapped gas leading to decreased venous return, decreased visceral perfusion and splinting of the diaphragm with consequent circulatory and respiratory compromise (12) and may even lead to cardiopulmonary arrest depending on the magnitude of the intraabdominal pressure (13). Since the first recorded case of tension pneumoperitoneum following resection of a pharyngeal diverticulum (14), there have only been a handful of case reports or small case series (15). Various iatrogenic procedures are responsible for this complication including positive-pressure ventilation, gastric rupture due to improper cardiopulmonary resuscitation, gastric or duodenal ulcer perforation and endoscopy. (16–19).

Although Minimum pneumoperitoneum is common after percutaneous endoscopic gastrostomy (PEG) tube insertion, tension pneumoperitoneum is rarely reported as a complication of the procedure. WDC Keale et al. (28) report a case of an 8-year-old microcephalic boy with spastic quadriplegia had longstanding swallowing incoordination who developed tension pneumoperitoneum and compartment syndrome immediately after insertion of percutaneous endoscopic gastrojejunostomy, which relieved by emergency decompression. They attributed the tension pneumoperitoneum to excessive leakage of air around the gastrostomy balloon during passage of the endoscope into the distal duodenum. Iscan Y et al (29) reported another case of pneumoperitoneum and with subcutaneous emphysema in a 45-year-old woman, who was diagnosed as having Neuro-Bechet's disease with swallow dysfunction happened 15 days after PEG tube insertion for long-term enteral feeding. Similarly, Bunni Jet al (30) reported a case of abdominal compartment syndrome caused by tension pneumoperitoneum in a fit 52-year-old experienced female scuba diver following a routine training dive to 27m.

Percutaneous radiological gastrostomy is well established as safe, reliable and effective alternative to PEG especially in cases in which passing an endoscope is technically difficult, with few minor complications such as superficial stomal infection, minor leakage around the tube, and few major complications such as peritonitis, bleeding, deep stomal infection, aspiration, displacement of the tube requiring a repeat procedure, and sepsis. (31)

Although simple and complicated pneumoperitoneum are well-recognized complication of PEG tube insertion they are rarely described as complications of percutaneous radiological gastrostomy, to our knowledge this case is the first case of tension pneumoperitoneum complicating radiologically inserted gastrostomy. Although the exact mechanism of our patient's TP is not clearly recognized, we think of that two factors contributed significantly to its development. The first factor was a large amount of air swallowed by mouth breathing secondary to the patient neurological status. The second factor was the use of pigtail-retained feeding tube without tube gastropexy with failure to attach or fix the stomach to the anterior abdominal wall. We also believe that this complication should have been prevented by using a balloon-retained or mushroom-retained tube as pushing the inflated balloon or the mushroom against the abdominal wall would have sealed the gastrotomy opening and prevented air leakage. In fact, some authors (32-34) advocate routine use of mushroom-retained tubes particularly in stroke patients who require long-term feeding and who might attempt to pull the tube out as a result of cognitive function.

Tension pneumoperitoneum is live threatening condition. Early diagnosis and prompt decompression is lifesaving. Diagnosis depends on high index of suspicious. The condition should be suspected in all patients who developed Hemodynamical and respiratory
compromise in addition to increasingly tympanic abdominal distension after endoscopy or abdominal percutaneous radiological procedures. The diagnosis can firmly be confirmed by plain radiographs of the chest and abdomen.

An erect chest x-ray is probably the most sensitive plain radiograph for the detection of free intraperitoneal air which shown the Dome sign demonstrated by the presence of bilateral dark crescent of gas under both hemidiaphragm. (35)

Massive free intraperitoneal air may demonstrate cupola sign which refers to non-dependent gas that rises within the abdominal cavity of the supine patient to accumulate underneath the central tendon of the diaphragm in the midline. It is seen as lucency overlying the lower thoracic vertebral bodies with well-defined superior border and ill-defined inferior margin. (36)

Multiple signs of free intraperitoneal air were described on supine abdominal radiographs including the double wall sign also known as Riegler sign which it is a sign of pneumoperitoneum with gas outlining both sides of the bowel wall result from presence of extra and intra luminal gas, usually seen when large amounts of free gas, >1000 mL, present within the peritoneal cavity, the falciform ligament sign (also called the Silver sign) is a sign seen with a pneumoperitoneum resulting from outlining of the falciform ligament with free intraperitoneal gas in a supine patient and the football sign (the intraperitoneal outlines the abdominal cavity, the falciform ligament appears like the laces of a football). (37)

CT scan is considered superior to simple plain radiograph in detecting the presence of pneumoperitoneum (38) and usually capable of demonstrating the presence of intra-abdominal free air in patients who had no evidence of pneumoperitoneum on chest radiograph. (25) but is usually not needed for confirmation of the diagnosis in cases of tension pneumoperitoneum as it may delay abdominal decompression.

Management of tension pneumoperitoneum like management of tension pneumothorax is immediate decompression which can be lifesaving. Decompression can be achieved by percutaneous needle decompression or surgery. Many authors advocate (1, 15, 39) percutaneous needle decompression either as a bridge to definitive surgical treatment or as part of conservative management in patients who are poor surgical candidates.

Summary

The percutaneous radiological gastrostomy (PRG) was devised as an alternative to PEG for enteral feeding in cases in which passing an endoscope is difficult or risky. Although few cases of tension pneumoperitoneum were reported as complication of PEG tube insertion, it was not reported as complications of percutaneous radiological gastrostomy. Appropriate selection of gastrostomy tube together with fixation of the stomach to the anterior abdominal wall during and after the procedure is important for prevention of this complication. Tension pneumoperitoneum is live threatening condition. Early diagnosis and prompt decompression are considered as lifesaving.

Conflict of Interest: Non-Declared

References Références Referencias

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The Role of 2D Speckle Tracking Echocardiography in Early Detection of Left Ventricular Dysfunction in Type II Diabetic Patients

By Mahmoud Shawky Abd El Moneum
Benha University

Abstract- Aim: The Early detection of diabetic heart disease is important for the timely interventions resulting in the prevention for the future development of heart failure. Left ventricular (LV) systolic dysfunction may be identified by a reduction in longitudinal function, which can be assessed using 2D speckle tracking echocardiography (STE).

Methods and Results: To determine longitudinal, radial, and circumferential function, three LV short-axis and three LV apical views were acquired in 100 asymptomatic diabetic patients with normal LV ejection fraction (EF) and 25 age-matched healthy volunteers. Using 2D strain software, end-systolic longitudinal strain (LS), radial strain (RS), and circumferential strain (CS) were measured in 18 LV segments. No significant differences in LVEF were noted between two groups. Diabetic patients had more advanced diastolic dysfunction and increased LV mass compared with normal subjects. Basal, middle, and apical LSs were significantly lower in diabetic patients compared with control subjects, with 43% (43/100) of the diabetic patients showing abnormal global LS values (cut-off value: 217.2, mean ± 2SD in control subjects).

Keywords: left ventricle; speckle tracking; longitudinal function; strain; diabetes mellitus.

GJMR-I Classification: FOR Code: WG 141.5.E2
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Conclusion: In addition to diastolic dysfunction, LV longitudinal dysfunction is preferentially impaired and frequently observed in asymptomatic diabetic patients with normal LVEF. The decrease in LS correlated with duration of diabetes. 2D speckle tracking echocardiography (STE) has the potential for detecting subclinical LV systolic dysfunction and might provide useful information on the risk stratification in an asymptomatic diabetic population.

Keywords: left ventricle; speckle tracking; longitudinal function; strain; diabetes mellitus.

I. Introduction

Diabetes mellitus (54 males and 46 females: mean age 63 ± 12 years). All patients had normal LVEF with no regional wall motion abnormalities on 2D echocardiography. Exclusion criteria included a history of coronary artery disease, the presence of moderate-to-severe valvular heart disease, and/or significant rhythm disturbances. We also enrolled 25 healthy control subjects (15 males and 10 females: mean age 62 ± 11 years) from our database for normal subjects. Healthy subjects were predominantly hospital employee or their relatives and/or friends. Because ageing affects diastolic function, we selected control subjects in order to adjust the same range of age. The Ethics Committee of the hospital approved the protocol and informed consent was obtained in every subject.

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b) Echocardiography

The Echocardiography was performed using a commercially available ultrasound equipment (M3S probe, Vivid S7). All 2D grey-scale echocardiographic images were obtained using second harmonic imaging. LV volumes and EF was measured using the modified Simpson method from the apical four- and two-chamber views. For the assessment of LV RS and CS, three LV short-axis planes were acquired at the basal, middle, and apical levels of the LV at high frame rates (range: 67–92 frame/s; mean 81±5 frame/s). Care was taken to ensure that the basal short-axis views were obtained at the level of the mitral valve, the middle planes at the level of the papillary muscles, and apical planes distal to the papillary muscles. For LS assessment, three LV apical views, apical four-chamber, two chamber, and long-axis views were acquired at high frame rates (range: 59–82 frame/s; mean 72±6 frame/s). In each plane, three consecutive cardiac cycles were acquired during a breath hold and digitally stored in a hard disk for off-line analysis. In order to measure the timing of cardiac events, LV inflow and outflow velocities were recorded using pulsed-wave Doppler echocardiography. Mitral annular velocity at the septal corner of the mitral annulus was also recorded to determine peak systolic, early diastolic, and late diastolic annular velocities.

c) Two-dimensional speckle tracking analysis

By using commercially available 2D strain software (Echopac PC, version 6.0), the endocardial border in the end-systolic frame was manually traced. A region of interest was then drawn to include the entire myocardium. The software algorithm automatically segmented the LV into six equidistant segments and selected suitable speckles in the myocardium for tracking. The software algorithm then tracked the speckle patterns on a frame-by-frame basis using the sum of absolute difference algorithm. Finally, the software automatically generated time-domain LV strain profiles for each of the six segments of each view, from which end-systolic strain was measured. The average value of strain at each level (basal, middle, and apical) and global strain obtained from averaging the strain values of 18 LV segments was calculated. We also evaluated longitudinal PSS. From time-domain LS waveforms throughout the cardiac cycle, we measured strain at end-systole (Lses) as well as post-systolic peak LS (LSpss). The post-systolic index (PSI) was calculated as ((LSpss 2 Lses)/Lses) x 100 (%) in each segment, and these values displayed in a parametric bull’s eye map (Figure 1). Whenever PSS was not present, PSI value of 0 was given.

Figure 1: Measurements of strain and post-systolic index. Upper three panels show longitudinal regional strain curve of six segments from the apical four-chamber, two-chamber, and long-axis views in a diabetic patient. The vertical line denotes aortic valve closure. In addition to longitudinal strain values at end-systole, post-systolic peak longitudinal strain was measured, if the regional strain curve reached its peak after the aortic valve closure. Lower panels show parametric image of end-systolic stain and post-systolic index. Note that post-systolic index was observed in the basal part of the myocardium.
**Statistical analysis**

Our study was designed with 90% power to detect a significant difference in global LS between diabetic patients and control subjects with a \( \frac{1}{2} 0.05 \). A difference in global LS between two groups of 3.0% was defined as clinically important, with an estimated SD of 3.0%. Due to reliability of multivariate analysis in diabetic patients, we enrolled 100 diabetic patients. Data were expressed as mean values ± SD or median values (interquartile range). Frequencies were expressed as percentages. All statistical analysis was carried out using commercially available statistical software (JMP, version 7.0 SAS). Differences in continuous variables between both groups were evaluated using paired or unpaired t-tests. Categorical variables were compared using Fisher’s exact test or \( \chi^2 \) test whenever appropriate. Linear regression analysis was used to investigate the relation between two parameters. Univariate and multivariate analyses were performed to determine independent predictors between LS and clinical and echocardiographic parameters. A \( P \)-value of 0.05 was considered significant.

**III. Results**

**a) Clinical and standard echocardiographic characteristics**

Table 1 shows the clinical characteristics of both groups. The mean diabetic duration was 8.7 years. Table 2 shows standard echocardiographic parameters. Although LVEF was not different between groups, LV mass index, relative wall thickness, and left atrial volume index were significantly higher in diabetic patients. Peak systolic and early diastolic annular velocity (E0) was significantly lower in the diabetic group, resulting in a higher E/E0 compared with control subjects.

| Table 1: Clinical characteristics of diabetic patients and control subjects |
|----------------------------------|---------------------|-----------------|
| **Diabetic patients** \( (n = 100) \) | **Control subjects** \( (n = 25) \) | **P-value** |
| Age (years) | 63 ± 12 | 62 ± 11 | NS |
| Sex, male/female (N) | 54/46 | 15/10 | NS |
| BSA (m²) | 1.60 ± 0.19 | 1.61 ± 0.15 | NS |
| HR (bpm) | 73 ± 13 | 67 ± 9 | <0.05 |
| HTN (N) | 47 | 0 (0) | <0.001 |
| Hyperlipidemia (%) | 47 (47) | 8 (32) | NS |
| Smoker (%) | 49 (49) | 6 (24) | <0.05 |
| Diabetic treatment | | | |
| Insulin (%) | 51 (51) | N/A | |
| SU (%) | 49 (49) | N/A | |

BSA, body surface area; DM, diabetes mellitus; HbA1c, haemoglobin A1c; HL, HR, heart rate; HTN, hypertension; SU, sulfonyl urea.

| Table 2: Standard echocardiographic data |
|-------------------------------------|---------------------|-----------------|
| **Diabetic patients** \( (n = 100) \) | **Control subjects** \( (n = 25) \) | **P-value** |
| IVS (mm) | 11.2 ± 1.6 | 9.4 ± 0.9 | <0.001 |
| PW (mm) | 10.8 ± 1.5 | 9.5 ± 1.2 | <0.001 |
| LVDd (mm) | 46 ± 6 | 44 ± 4 | NS |
| LVDs (mm) | 29 ± 5 | 28 ± 3 | NS |
| LVEDV (mL) | 71.7 ± 20.7 | 81.2 ± 17.7 | <0.05 |
| LVESV (mL) | 26.2 ± 11.3 | 29.1 ± 7.6 | NS |
| LVEF (%) | 64.4 ± 7.2 | 64.2 ± 5.7 | NS |
| LVMI (M-mode) (g/m²) | 115 ± 30 | 80 ± 16 | <0.001 |
| RWt | 0.49 ± 0.10 | 0.43 ± 0.05 | <0.005 |
| LAVI (mL/m²) | 38.1 ± 11.4 | 26.7 ± 4.6 | <0.005 |
| E (cm/s) | 64 ± 17 | 66 ± 14 | NS |
| A (cm/s) | 82 ± 20 | 67 ± 13 | <0.001 |
| DcT (ms) | 243 ± 68 | 215 ± 64 | NS |
| E / A | 0.8 ± 0.3 | 1.0 ± 0.3 | <0.05 |
| IVCT (ms) | 46.3 ± 27.7 | 36.3 ± 21.6 | NS |
| IVRT (ms) | 104.7 ± 23.7 | 88.3 ± 14.7 | <0.005 |
| S’ velocity (cm/s) | 6.9 ± 1.8 | 8.0 ± 1.5 | <0.05 |
| E’ velocity (cm/s) | 5.6 ± 2.0 | 7.5 ± 1.9 | <0.001 |
| E / E’ | 12.9 ± 5.1 | 9.1 ± 2.4 | <0.005 |

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A, mitral late diastolic peak velocity; DcT, deceleration time of the E-wave velocity; E, mitral early diastolic velocity; E′, peak mitral annular velocity during early diastole; IVCT, isovolumic contraction time; IVRT, isovolumic relaxation time; LAVI, left atrial volume index; LVDD, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; LVEDV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVMi, left ventricular mass index; NS, not significant; PW, posterior wall; RWT, relative wall thickness; S′, peak mitral annular velocity during systole.

b) End-systolic strain values

The global and regional three-principal strain values are shown in Figure 2. Global and regional LSs at the base-, mid-, and apical-LV levels were significantly lower in diabetic patients compared with control subjects. Global LS in control subject was 220.8±1.8. These data were used to establish abnormal cut-off value of global LS. This was calculated as the value of the mean ± 2SD. Using the cut-off value of 217.2, 43% (26/60) of the diabetic patients showed abnormal global LS value. CS at the base- and the mid-LV levels did not differ between groups. However, CS at the apical level was significantly lower in diabetic patients, resulting in a significant reduction in global CS. Diabetic patients had also significantly lower regional RS at the basal level and global RS compared with control subjects. Similar results were obtained when diabetic patients with LV hypertrophy on 2D echocardiography were excluded from the analysis. No significant correlation was noted between LVEF and global LS (r = -0.05, P = NS) or RS (r = -0.24, P = NS). A weak albeit significant negative correlation was noted between LVEF and global CS (r = -0.38, P < 0.005).

Figure 2: Showing global and regional strain values of diabetic patients and control subjects. Numerical values are represented as mean ± SD. In each box plot, upper and lower bars represent 90th and 10th percentiles. Top of the box represents 75th percentile, line in the box median value and bottom of the box means 25th percentile.
Univariate analysis revealed that the reduction of global LS was independently associated with E0 (P, 0.0001), relative wall thickness (P, 0.0001), duration of diabetic disease (P \( \frac{1}{4} \) 0.0006), albuminuria (P \( \frac{1}{4} \) 0.0037), and E-wave velocity (P \( \frac{1}{4} \) 0.0257). No correlation was noted between the reduction of global LS and fasting blood glucose (P \( \frac{1}{4} \) 0.7489) or glycosylated haemoglobin (P \( \frac{1}{4} \) 0.7524). Multivariate linear regression analysis demonstrated that diabetic duration was the only independent predictor for LS reduction (t \( \frac{1}{4} \) 2.22, P \( \frac{1}{4} \) 0.0313). When dividing diabetic patients into two groups according to the duration of disease (.5 and .5 years), global LS was significantly lower in the diabetic group with longer disease duration (216.7+3.0) compared with the short diabetic duration group (218.2+1.9, P , 0.05). Although no significant differences in global RS (46.0+11.7 vs. 44.9+11.7) and CS (223.1+3.9 vs. 222.2+3.5) were noted, RS at the apical level (38.4+18.7 vs. 27.5+16.0, P , 0.05) and CS at the middle level (223.5+4.2 vs. 221.2+3.6, P , 0.05) were significantly higher in the diabetic group with prolonged disease duration compared with the short duration group.

c) **Post-systolic shortening**

**Figure 3** shows the PSS indices in both groups. The PSI value was significantly larger in diabetic patients compared with control subjects. PSI was significantly larger at the basal level compared with the middle or apical LV levels in both groups. Parametric PSS maps revealed that the distribution did not correlate with the perfusion territory of any coronary artery. PSI values significantly correlated with endsystolic LS in all subjects (n \( \frac{1}{4} \) 85, r \( \frac{1}{4} \) 0.69, P, 0.001) as well as diabetic patients (n \( \frac{1}{4} \) 60, r \( \frac{1}{4} \) 0.64, P, 0.001).

![Figure 3: Post-systolic index between the two groups](image)

**IV. Discussion**

The major findings of this study can be summarized as follows: (i) although global RS, CS, and LS were significantly reduced in diabetic patients compared with age-matched control subjects, the reduction in LS was more prominent and evenly distributed throughout the LV. (ii) The duration of diabetic disease was the only independent predictor for the decrease in LS. (iii) Diabetic patients had more evidence for PSS index, and its distribution did not match the vascular territory of any coronary artery.

a) **Two-dimensional strain**

Identification of early manifestations of diabetic heart disease would allow the institution of timely medical interventions to prevent the development of heart failure. Although diastolic dysfunction has been described as an early stage in diabetic heart disease progression in patients with normal LVEF,[5,6] isolated diastolic dysfunction is usually rare,[7] and when present, it often associated with subclinical systolic dysfunction. Systolic dysfunction might be initially apparent in the longitudinal direction, because subendocardial fibres, which are the ones more
vulnerable to myocardial ischaemia and fibrosis, are longitudinally oriented.[8,10] Several studies have demonstrated that systolic longitudinal dysfunction can be identified using tissue Doppler imaging in patients with hypertension,[18] diabetes,[19,20] and diastolic dysfunction.[21] However, this method provides information in a single direction from a fixed transducer position. This method is also dependent on the angle between the beam and myocardial motion. In contrast, 2DSTE has the advantage that it allows the measurement of all principal LV strains in an angle independent manner, thus eliminating the major limitation of tissue Doppler imaging. Similar to previous tissue Doppler studies,[19,20] we observed that global and regional LSs were significantly reduced in diabetic patients, with 40% of the patients showing abnormal LS values compared with the normal range obtained from our control group of age-matched subjects. In addition, global RS and CS were also reduced in diabetic patients, a finding which is in agreement with a previous magnetic resonance imaging study.[22] On the contrary, Fang et al.,[11] using tissue Doppler imaging reported that reduced longitudinal function was compensated by the augmentation of radial function in diabetic patients. Differences between study populations and in the method of measuring strain could have accounted for the discrepancies between Fang’s and our study. Our results suggest that abnormal function is more widespread than just in the longitudinal direction in diabetic patients. We found that the reduction in global LS was independently associated with diabetic duration as well as early diastolic indices (E-wave velocity and E′), relative wall thickness, and albuminuria. Significant correlation between global LS and E′ confirms the link between systole and diastole, which has been confirmed in previous studies.[23,24] Albuminuria is independently associated with systolic and diastolic dysfunction in diabetic patients.[25,26] The present study showed that diabetic duration was the only independent predictor for the reduction in LS. This highlights the relationship between long-term hyperglycaemia and the impairment of LS. Although global LS was reduced, regional RS and CS were paradoxically increased in diabetic patients of long-term duration. This augmentation in regional RS and CS might reflect a compensation to maintain LVEF in diabetic patients with a long history of disease.

b) Post-systolic shortening

Myocardial shortening after aortic valve closure, i.e. PSS, has been suggested as a sensitive marker of regional myocardial dysfunction.[16,17] However, PSS may also occur in healthy subjects. To discriminate between pathological and physiological PSS, Voigt et al.,[17] described that the timing and the magnitude are different between these two situations. The present study showed that PSS was significantly larger in diabetic patients compared with control subjects. Pathological PSS is usually associated with a reduction in systolic strain. The finding that PSI was negatively correlated with LS in our study supports this concept. Thus, we propose that PSS with reduced LS is a marker of myocardial dysfunction in diabetic patients with preserved LVEF. Interestingly, the distribution of PSS in both diabetic patients and control subjects was mainly observed in the basal myocardium. We also noted that its distribution did not correlate with the vascular territory of the coronary arteries. Although the precise mechanism of why PSS is preferentially observed in the basal myocardium is unknown, PSS observed in this study is not related to myocardial ischaemia induced by epicardial coronary artery stenosis.

c) Study limitations

The study size was relatively small. Thus, our results cannot be extrapolated to the general diabetic population. The majority of diabetic patients had concomitant hypertension, which also affects longitudinal function. However, exclusion of hypertensive diabetic patients would produce significant bias in our results. Diabetic patients were considered to have a low probability of coronary artery disease based on clinical grounds and normal resting echocardiography.

V. Conclusions

LVEF is not a sensitive indicator for the detection of subclinical systolic dysfunction in our study. Diabetic duration was the only independent predictor for the reduction of global LS. 2DSTE has the potential for detecting subclinical LV systolic dysfunction, and it might provide useful information for the risk stratification of an asymptomatic diabetic population.

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Cardiac Effects of (−)-Epigallocatechin on Isolated Rat Hearts

By Loipa Galán Martínez, Idalia Herrera Estrada & Alicia Fleites Vázquez

Instituto de Cardiología y Cirugía Cardiovascular

Abstract- (−)-Epigallocatechin is a flavonoid found in many plants, especially in tea. The consumption of flavonoid-rich foods tends to reduce the risk of cardiovascular diseases and this has been attributed to nonspecific activities such as antioxidant, anti-atherosclerotic, and anti-inflammatory properties. But, the direct actions of (−)-epigallocatechin on cardiac muscle still not known. This study evaluated the effects of (−)-epigallocatechin on electrical and contractile activities of isolated rat hearts. Surface electrogram and force of contraction were recorded in isolated rat hearts in control and with increasing concentrations of (−)-epigallocatechin from 0.001 to 3 μM. (−)-Epigallocatechin tended to prolong the QRS interval, but this effect is significant only at the highest concentration studied (3 μM). QTc was not significantly affected by the flavonoid. The actions of this flavonoid on RR interval were mild and statistically significant since 0.03 μM. (−)-Epigallocatechin decreased the force of contraction of isolated rat hearts with an IC50 of 0.03 μM. This flavonoid has direct actions on rat cardiac muscle. Keywords- Cardiovascular; (−)-Epigallocatechin; Flavonoids.

GJMR-I Classification: FOR Code: WG 170

Strictly as per the compliance and regulations of:
Cardiac Effects of (−)-Epigallocatechin on Isolated Rat Hearts

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I. INTRODUCTION

Catechins are one group of natural polyphenols found in many plants, especially in green tea (leaves of Camellia sinensis) (1-3). The four main catechin derivatives mainly found in green tea include the isomers epicatechin, (−)-epicatechingallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG) (3). EGC is a flavan-3-ol containing a (−)−epigallocatechin gallate (10). The present work evaluated the possible direct effects of EGC on electrical and contractile activities of rat isolated rat hearts.

II. MATERIALS AND METHODS

a) Animals

Male adult (7-8 weeks) Wistar rats were brought from the National Center for Laboratory Animal Reproduction (CENPALAB; La Habana). Before the experiments, animals were for seven days adapted to laboratory conditions (controlled temperature 25 ± 2°C, relative humidity 60 ± 10%, and 12 h light/dark cycles). Tap water and standard diet for rodents supplied by CENPALAB were freely provided. All procedures fulfilled with the European Commission for the use and care of laboratory animals. The Committee for Animal Care in Research of the Center (No. 08-2012, folio 73, book 01, 2012) approved the present study.

b) Isolated hearts

As previously reported (11), under pentobarbital anesthesia rat hearts were removed and placed in cold Tyrode (see below). Hearts were carefully dissected, mounted on a Langendorff column and perfused at constant flow (10 mL/min) with a Tyrode solution of the following composition (mmol/L): 140 NaCl, 2.5 KCl, 0.5 MgCl2, 2 CaCl2, 10 Tris- hydroxymethyl amino methane, 10 Glucose (pH =7.4, gassed with O2; T = 35°C). On the ventricular epicardium was placed a bipolar platinum recording electrode to record the surface electrocardiogram. Another bipolar platinum electrode was placed near the atrioventricular ring and was connected to an electronic stimulator. To record the force of contraction (FC), the cardiac apex was fixed to a force-displacement transducer with a surgical 6-0 silk thread. Surface electrocardiogram and FC values were recorded at the heart rate and a fixed stimulus rate (500-ms RR interval).

c) ECG and chemicals

Stock solutions of ECG were prepared in ethanol, and diluted in the bathing solution on the day of the experiment. All chemicals were from Sigma Aldrich.
d) Statistical analysis

Means and standard errors of means expressed the results. Student’s t-test evaluated the statistical significance for paired samples, previously checked that the data complied with the premise of normality. Differences were considered statistically significant for \( p < 0.05 \). The graphics and the statistical processing were done using the software OriginPro 8 SRO v8.0724 (MA, USA).

![Chemical structure of (-)-Epigallocatechin](image)

**Figure 1:** Chemical structure of (-)-Epigallocatechin

**III. RESULTS AND DISCUSSION**

The corrected QT (QTc) interval of the surface electrocardiogram (\( QTc = QT/\sqrt{RR} \)) was not significantly affected by EGC at concentrations from 0.001 to 3 \( \mu M \) (Table 1).

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<th>Table 1: Effects of different concentrations of (-)-Epigallocatechin (EGC) on QTc, QRS, and RR interval</th>
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<td>Control</td>
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<td>EGC 1 ( \mu M )</td>
</tr>
<tr>
<td>EGC 3 ( \mu M )</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \) vs. Control

These results should be possible because this flavonoid could exert multiple actions on different ionic channels, resulting in an apparent absence of effects on QT interval of the cardiac surface electrogram. As a fat, catechins modulate several ionic channels (12-15).

EGC showed a tendency to increase QRS interval of the surface electrocardiogram, but only at the highest concentration studied (3 \( \mu M \)) this increase was statistically significant (\( p < 0.05 \)) (Table 1). EGCG, catechin structurally related to EGC, at 30 \( \mu M \) prolonged QRS interval in isolated spontaneously beating guinea pig hearts (15). The QRS wave is dependent on sodium channel activity, Kang et al., 2010 showed that EGCG inhibited the cloned human cardiac sodium channel Nav1.5 in a dose-dependent manner with 45.7 ± 6.9 % inhibition at 100 \( \mu M \) (15). EGCG reduced the amplitude of voltage-gated sodium channel current in a concentration-depend manner in the range of 0.1 - 400 \( \mu M \) in rat hippocampal CA1 neurons (13).

On the other hand, EGC prolonged the RR interval of surface electrocardiogram and this increase was statistically significant (\( p < 0.05 \)) since 0.03 \( \mu M \) (Table 1).

EGCG at 30 \( \mu M \) did not affect heart rate of guinea pig hearts (15). Green tea extract used with dietary supplements did not alter heart rate (16). Other study concluded that Camellia sinensis has effect on heart rate, it decreases the heart rate in normotensive female individuals and increases the heart rate in the normotensive male individuals (17).
Figure 2: Concentration-response curves for the inhibition of force of contraction by EGC. Experimental data (n = 6 for each point) were fitted to a Hill function.

Although further studies are needed to see if EGC has any direct effect on calcium channels, the decrease of force of cardiac contraction by EGC should be at least partly due to an inhibition of calcium channels.

The L-type calcium channel was inhibited by 20.8% at 30 µM by EGCG, reached a maximum of 37.1 ± 4.2% at a concentration of 100 µM (15). Tadano et al., 2010 reported that EGC had no significant effects on cardiac myofilament Ca2+-sensitivity. However ECG and EGCG were found to decrease Ca2+ sensitivity, they were Ca2+ desensitizers acting through binding to cardiac troponin C (18).

At concentrations within the same range at which similar flavonoid EGCG have vasorelaxant effects related to the inhibition of Ca2+ influx in smooth muscle cells (19), in the present results, EGC concentration-dependently relaxed with almost equal effectiveness the contraction of rat hearts.

On the strength of these results, the physiological relevance of the decrease of force of cardiac contraction by EGC can be asserted by considering the data available on the in vivo level of the related catechin EGCG ([EGCG] = 0.3–7.5 µM in the blood of green tea consumers (20)).

Three-month supplementation with green tea capsules decreased systolic (SBP) and diastolic blood pressure (DBP) by four mmHg in obese hypertensive (21) but not obese subjects (22). A recent metaanalysis which included eleven trials concluded that short-term consumption (>6 months) of black tea could decrease SBP and DBP by 1–2 mmHg and green tea by three mmHg (23).

Moreover, treatment with EGCG protected rat hearts from ischemia/reperfusion in vivo; this could suggest that EGCG is beneficial for the treatment of reperfusion-induced myocardial damage (24-25).

IV. Conclusions

The present study revealed that EGC has direct cardiac effects. The results presented here confirm the role of tea catechin EGC, as a precursor for the development of novel drugs for the treatment of cardiovascular disorders.

REFERENCES Références Referencias


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13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.
20. **Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. **Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. **Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. **Upon conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

**Informal Guidelines of Research Paper Writing**

**Key points to remember:**
- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

**Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

**The introduction:** This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

**The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

**General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

**To make a paper clear:** Adhere to recommended page limits.
Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:
Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:
The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.
The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study’s tentative purpose and how it meets the declared objectives.

**Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

**Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

**Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

**Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

**Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer’s interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

**What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.
Results:
The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:
- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:
- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:
As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:
If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:
The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

**Approach:**

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

**The Administration Rules**

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

*Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.*

*Segment draft and final research paper:* You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

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**BY GLOBAL JOURNALS**

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<table>
<thead>
<tr>
<th>Topics</th>
<th>Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-B</td>
</tr>
<tr>
<td><strong>Abstract</strong></td>
<td>Clear and concise with</td>
</tr>
<tr>
<td></td>
<td>appropriate content, Correct</td>
</tr>
<tr>
<td></td>
<td>format. 200 words or below</td>
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<tr>
<td><strong>Introduction</strong></td>
<td>Containing all background</td>
</tr>
<tr>
<td></td>
<td>details with clear goal and</td>
</tr>
<tr>
<td></td>
<td>appropriate details, flow</td>
</tr>
<tr>
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<td>specification, no grammar</td>
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<tr>
<td></td>
<td>and spelling mistake, well</td>
</tr>
<tr>
<td></td>
<td>organized sentence and</td>
</tr>
<tr>
<td></td>
<td>paragraph, reference cited</td>
</tr>
<tr>
<td><strong>Methods and</strong></td>
<td>Clear and to the point with</td>
</tr>
<tr>
<td><strong>Procedures</strong></td>
<td>well arranged paragraph,</td>
</tr>
<tr>
<td></td>
<td>precision and accuracy of</td>
</tr>
<tr>
<td></td>
<td>facts and figures, well</td>
</tr>
<tr>
<td></td>
<td>organized subheads</td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td>Well organized, Clear and</td>
</tr>
<tr>
<td></td>
<td>specific, Correct units with</td>
</tr>
<tr>
<td></td>
<td>precision, correct data, well</td>
</tr>
<tr>
<td></td>
<td>structuring of paragraph, no</td>
</tr>
<tr>
<td></td>
<td>grammar and spelling</td>
</tr>
<tr>
<td></td>
<td>mistake</td>
</tr>
<tr>
<td><strong>Discussion</strong></td>
<td>Well organized, meaningful</td>
</tr>
<tr>
<td></td>
<td>specification, sound</td>
</tr>
<tr>
<td></td>
<td>conclusion, logical and</td>
</tr>
<tr>
<td></td>
<td>concise explanation, highly</td>
</tr>
<tr>
<td></td>
<td>structured paragraph</td>
</tr>
<tr>
<td></td>
<td>reference cited</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>Complete and correct format,</td>
</tr>
<tr>
<td></td>
<td>well organized</td>
</tr>
</tbody>
</table>
## INDEX

**A**
- Amplatzer · 7
- Aneurysm · 2, 3
- Angiogram · 4
- Aortocaval · 2, 3, 5, 7, 8

**B**
- Barotrauma · 20
- Bifurcated · 3, 5, 7

**E**
- Endoleak · 3, 5, 8, 10

**F**
- Falciform · 18
- Flavonols · 37
- Flexion · 12

**G**
- Gastrograftin · 14

**H**
- Hippocampal · 33, 37

**I**
- Intraperitoneal · 14, 15, 18

**N**
- Normoalbuminuric · 29
- Normotensive · 28, 29, 33

**P**
- Paresthesia · 3
- Percutaneously · 12

**R**
- Reperfusion · 35, 38
- Retroperitoneal · 4

**S**
- Syphilitic · 3

**T**
- Troponin · 35, 38
- Tympanic · 15, 18

**U**
- Univariate · 25, 27

**V**
- Vasorelaxant · 35