Long term outcome of Video-Assisted Thoracoscopic Sympathectomy at thoracic level (R2-3) versus (R2-4) on Plantar Hyperhidrosis associated with Primary Palmar Hyperhidrosis

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Abstract

Background: Hyperhidrosis is unnecessary sweating afar the body needs for cooling down the body temperature. It affects nearly 2-3% of the population with equal gender predominance. Both medical and surgical interventions have been utilized to treat primary hyperhidrosis. Over 80% of patients with palmar hyperhidrosis (PH) also experience plantar hyperhidrosis (PLH). An interesting observation in surgically treated patients with PH is that PLH symptoms tend to decrease after undergoing Video-assisted thoracoscopic surgery (VATS).

Objectives: The aim of the present study was to evaluate the efficacy, safety and drawbacks of VATS sympathectomy at thoracic levels (R2-3) versus (R2-4) on PLH associated with primary PH.

Patients and methods: this single center, parallel randomized controlled study involved 72 eligible patients presented with primary palmoplantar hyperhidrosis, where 36 patients underwent VATS sympathectomy at thoracic levels R2-3 (Group A) versus other 36 patients at thoracic level R2-4 (Group B). Both groups underwent bilateral two-port tubeless VATS sympathectomy with the use of electrocautery under general anesthesia in semi-fowler position.

Results: Following surgery, both groups showed improvement in PLH, but group B had a significantly higher incidence of improvement compared to group A. In group A, 77.7% of patients were satisfied, while in group B, 88.8% of patients reported satisfaction.

Conclusion: VATS sympathectomy is the preferred treatment for effectively curing PH. There is a significant correlation between VATS sympathectomy performed at the R2 to R4 levels and the alleviation of PLH.

Keywords: Palmar hyperhidrosis; Plantar hyperhidrosis; VATS, Sympathectomy.

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Introduction

Hyperhidrosis (HH) is excessive sweating beyond what the body needs to cool down the body temperature. It just affects 2-3% of the population with equal predominance between males and females. **(Wörle et al., 2007).** It affects different areas of the body, predominantly occurring in palms, axilla, sole, face, and inguinal region. It could affect one area alone or affect more than one area together. **(Wörle et al., 2007).**

The problem of this condition is that it may cause social stress and embarrassment to the patient that could affect his daily activity and may cause psychological disturbance. **(Kopelman et al., 2012)**

Hyperhidrosis is caused by unknown stimulation of sympathetic nervous system despite any stresses. It could be primary or secondary to an underlaying disease e.g., thyrotoxicosis. **(Kopelman et al., 2012)**

Over many years, different modalities, either medical or surgical, have been used for the treatment of primary hyperhidrosis. The surgical approach is now considered the gold standard with long term outcome via thoracic approach for palmar, axillary, and facial HH, or via abdominal approach for the plantar HH. **(Wörle et al.,2007; Kopelman et al., 2012)**.

In more than 80% of the cases, patients who present palmar hyperhidrosis will be associated with plantar hyperhidrosis. Palmar hyperhidrosis (PH) is usually the most important complaint, and that is why they undergo video-assisted thoracoscopy (VATS). **(de Campos et al.,2003).** VATS cutting, cauterizing, or clipping of the sympathetic trunk is treatment of choice for PH. **(Huang et al., 2019).** Patients can undergo VATS sympathectomy at any age, as long as suitable surgical instruments are available. **(Huang et al., 2019).**

Compensatory sweating (CS) is the most commonly unpredictable adverse effect, where sweating in part of the body increases and become hyperactive in order to compensate the other part, which becomes hypo-hidrostic or an-hidrotic after sympathectomy.

Lumbar sympathectomy is widely limited to the patients with vascular problems and this gives the upper hand to Radio Frequency over surgery for plantar hyperhidrosis as it is less invasive and is preferred by the patients. **(Racz and Stanton-Hicks, 2002)**.

A surgical link was observed on patients treated by VATS sympathectomy for PH cases and the improvement or diminishing of symptoms of PLH, which is difficult to be explained anatomically and physiologically. **(Wolosker, 2013)** An explanation was that the treatment of the PH decreases social stress through decrease in sympathetic stimulation and so improves the PLH. **(Reisfeld, R et al.,2002).** The aim of the present study was to evaluate the efficiency of video-assisted thoracoscopic sympathectomy at thoracic level (R2-3) versus (R2-4) on plantar hyperhidrosis associated with primary palmar hyperhidrosis.

Patients and methods

The study involved 72 eligible patients presented with primary palmoplantar hyperhidrosis treated by bilateral, bi-portal, and tubeless thoracoscopic approach under general anesthesia. All age groups and genders were included. The patients were divided into two equal groups. Thirty-six patients were treated with VATS sympathectomy at thoracic level R2-3 (Group A), and the other 36 patients were treated with VATS sympathectomy at thoracic level R2-4 (Group B). The groups were randomized using the closed envelope method.

We excluded cases with severe cardio-pulmonary insufficiency, presence of history of severe pleural diseases (empyema, pleuritis), previous thoracic surgery and sympathectomies, the inability to maintain adequate arterial oxygen saturation with single-lung ventilation or

CO_2 insufflation, and untreated hyperthyroidism.

This single center prospective randomized controlled study was conducted at the department of Cardiothoracic Surgery, Alexandria University Hospitals, in Alexandria, Egypt, from August 2021 to February 2022.

After approval of the institutional research board (IRB) and the medical ethics committee at Alexandria University was obtained. A written informed consent was obtained from all eligible patients, or their legal guardians enrolled in the study.

The primary outcome was to identify the thoracic level associated with an eminent relieving effect on planter hyperhidrosis that was measured by cure or improvement of planter sweating. The secondary outcome measures the degree of improvement of plantar hyperhidrosis through subjective evaluation of the pre- and post-operative Visual Analogue Scale (VAS) of ten degrees (**Abu Arab and Elhamami, 2021)** at three-time intervals after a week, after 6 months, and after 1 year in both groups. Complications such as lung injury, bleeding, and postoperative complications such as Horner's syndrome, compensatory hyperhidrosis, pneumothorax, chylothorax, hemothorax, intercostal neuralgia, and recurrence.

All eligible patients underwent VATS sympathectomy at semi-fowler position with the use of a double lumen endotracheal tube (ETT), or in the absence of a double lumen ETT, Co_2 insufflation is used on each side apart to produce lung collapse with a maximum pressure flow 8-12 mmHg.

A bi-portal incision of around 5 or 10 mm anterior axillary line at the fifth intercostal space used for the 5 or10 mm trocar to be used as the camera port (Karl-Storz, Germany) to inspect the pleural cavity and identify the sympathetic chain. Another incision of approximately 5 mm was made at the axillary region in the second intercostal space just below the pectoralis major muscle fold for placing a 5 mm trocar to introduce a hook or Maryland grasper.

Then, exposure and isolation of the sympathetic chain were achieved from the level of 2nd rib to the level of 3rd rib in (group A) **(Fig.1)** and from the 2nd to the 4th rib in (group B) **(Fig.2)**. Then, cauterization over the upper border laterally on each level according to the study group for cauterization of the nerve of Kuntz **(Cinà et al., 2007)**. The choice of sympathectomy (interruption) or sympathectomy (removal of the whole segment of the chain) was made as per the operating surgeon's preference. Both techniques were used in both study groups.

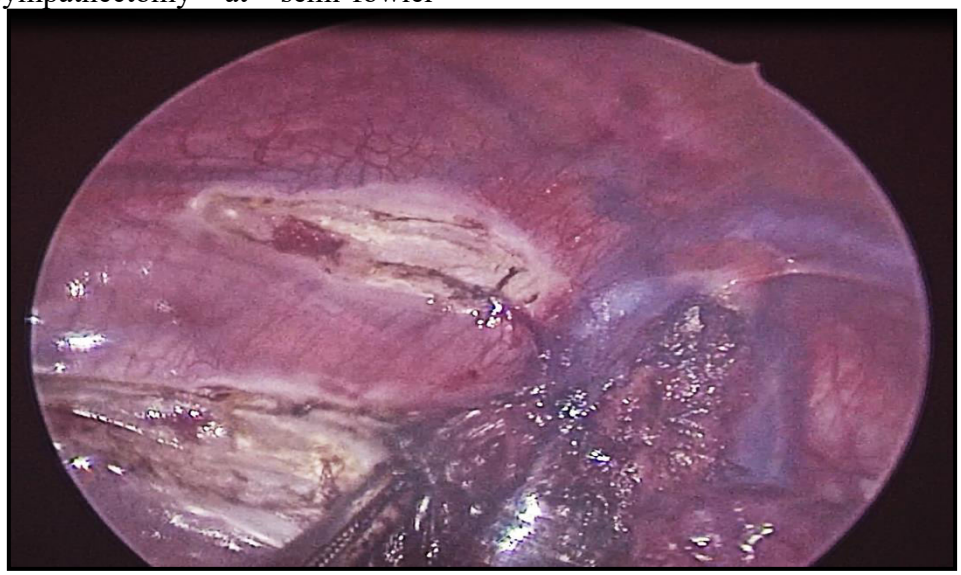

Fig.1. Cauterization of the sympathetic chain at R2 to R3 segments (Right side).

Fig.2.Cauterization of the sympathetic chain at R2 to R4 segments (Left side).

Haemostasis and deairing were then done using a silicon tube connected to an underwater seal device to allow lung expansion while maintaining positive pressure ventilation. The same procedure was done to the contralateral side in the same setting. Chest X- ray was performed two hours postoperatively and the patient was discharged home on the same day if the patient was clinically and radiologically free. **(Cinà et al., 2007)**

The study data was statistically analyzed. Based on literature **(Huang et al., 2019)**, a reported mean success rate of cured or improved planter hyperhidrosis following thoracoscopic sympathectomy at R2-R3 levels of 57% compared to 88.8% R3-R4 level. A least significant sample size of 72 patients allocated to two equal groups was calculated to have a study of 80% power with an alpha error of 5%, a beta error of 20%, and an effect size of (d= 0.67), (Critical t= 1.9944371) using A-Priori two tailed independent pairwise comparisons of dichotomous endpoint, between two independent groups using G*Power computer software **(Faul et al., 2013)**.

Statistical analysis

The study data were tabulated and analyzed using IBM SPSS software package version 24.0 (IBM Inc., Chicago, IL, USA). Qualitative data were depicted using numbers and percentages. Quantitative data were designated using interquartile range, mean, standard deviation (SD). Pairwise comparisons between distinct groups concerning categorical variables were checked using Chi-square test (χ2-test). When more than 20% of the cells expected count less than 5, correction for chi-square was conducted using Fisher's exact test or Monte Carlo correction. For normally distributed data, comparisons between two independent populations were done using t- test. For abnormally distributed data, the Mann-Whitney Z test and Wilcoxon signed ranks tests were used.

The confidence interval was set to 95% and the margin of alpha error accepted was set to 5%. So, the p-value was considered significant as the following: $P > 0.05$: Non-significant, $P \leq$

0.05: Significant, and P < 0.01: Highly significant.

Results

Group A (R2-3) had a mean age of 17±5.91 years; 52.8% were females. Group B (R2-4) had a mean age of 19.72 ± 5.77 years; 66.7% were males, with no statistically significant difference.

The operative time in group A ranged from 15 and 30 minutes (21.58± 3.47 mins) for both sides while in group B; it ranged from 18 and 35 minutes (22.64±4.05 mins) without statistically significant difference. one patient in each group (2.8%) had an intraoperative bleeding that was controlled intraoperatively by compression and cautery. One patient in group B (2.8%) was complicated by a unilateral air leak intraoperatively, which was managed by the insertion of an intercostal tube (ICT) connected to the underwater seal. This patient was followed-up for 24 hours by plain Chest X-ray-PA, clamping of ICT at first post-operative day then removal and discharge after a second plain Chest X-ray-PA.

In the post-operative complications, there was only one patient with superficial wound infection (2.8%) in group A. This patient was managed by frequently dressing with betadine and bacitracin spray. No patient in both groups showed Horner syndrome or recurrence.

As regards compensatory hyperhidrosis (CH), 12 patients in group A showed mild CH (33.3%), 2 patients showed moderate CH (5.6%), and no patient showed severe symptoms, where the total number of patients with CH formed 14 patients (38.9%). While in group B; 14 patients showed mild CH (38.9%), 4 patients showed moderate CH (11.1%) and no patient showed severe symptoms, with a total of 18 patients (50%) suffered CH.

According to the VAS-score for CH; where score zero was settled for the least symptoms and 10 for the severe symptoms; Group A; showed VAS-score of (1.06 ± 1.53) while it was (1.42±1.69) in group B.

Pre-operatively, the mean of the VAS-score for PH in group A was found to be 8.61 ± 1.20, while in group B; it was 8.61 ± 1.05. Post-operatively, the VAS-Score in both groups was followed up after one week, six months and one year.

In group A patients, the mean post-operative VAS-score for PH at one week was (1.28 ± 0.45), it was the same even after six months and one year follow-up after surgery. While in group B, the mean of the VAS-Score after one week of surgery was 1.44 ± 0.56, and it remains approximately at the same value after six months and one year following the with a mean VAS-Score of 1.39 ± 0.49. There was a change in group B in six months and 1 year as two patients (5.55%) changed their VAS-Score as they showed more improvement after 6 months and showed a higher level of satisfaction **(Table.1).**

Table.1. Degree of Pre- and post-operative palmar hyperhidrosis in both groups

PH VAS of 10	Group A (n = 36)	Group B (n = 36)	Test for sig.	P value
Preoperative				
Mean ± SD.	8.61 ± 1.20	8.61 ± 1.05	-	n/s
Min. – Max.	7.0-10.0	7.0-10.0		
1 Week postoperative				
Mean ± SD.	1.28 ± 0.45	1.44 ± 0.56	t =1.39	0.169
Min. – Max.	1.0 – 2.0	1.0-3.0		
6 Months postoperative				
Mean ± SD.	1.28 ± 0.45	1.39 ± 0.49	t =0.99	0.324

Min. – Max.	1.0 – 2.0	1.0-3.0		
1 Year postoperative				
Mean ± SD.	1.28 ± 0.45	1.39 ± 0.49	t =0.99	0.324
Min. – Max.	1.0-2.0	1.0-3.0		

*Statistically significant relation

All patients in both groups showed significant cure and satisfaction from the improvement of palmar hyperhidrosis with no recurrence.

In comparison to PH, PLH improvement following surgery in group B; showed high statistical significance value (p= 0.002) with a higher prevalence of PLH improvement in such patients; 26 (72.2%) patients in group B and two patients in group A (5.6%).

Pre-operatively, the mean of the VAS-score for PLH in group A patients was (6.11 ± 1.14), while group B; it was (5.97 ± 0.88). There was no significant statistical difference between both studied groups regarding the pre-operative VAS-Score for plantar hyperhidrosis.

Post-operatively, the VAS-score regarding the degree of PLH in the patients of the two groups was followed up after one week, six months and twelve months. In group A, there was no change during the follow up at the three intervals with where the mean of the measured VAS score was 6.08 ± 1.36, while in group B, the mean of the post-operative VAS score was 3.83 ± 1.61 and it showed no change at the three follow up intervals. It was noted that there was a high statistically significant difference between both means of the VAS -score of both regarding the improvement of PLH in group B (p =0.0001) **(Table.2).**

Table. 2. Degree of pre and post operative plantar hyperhidrosis in both groups on VAS-score

PLH VAS of 10	Group A (n = 36)	Group B (n = 36)	Test for sig.	P value
Preoperative				
Mean ± SD..	6.11 ± 1.14	5.97 ± 0.88	-	n/s
Mean ± SD.	4.0-9.0	5.0-8.0		
1 Week postoperative				
Mean ± SD.	6.08 ± 1.36	3.83 ± 1.61	t = 6.4	0.0001 *
Min. – Max.	2.0 – 9.0	2.0 – 7.0		
6 Months postoperative				
Mean ± SD.	6.08 ± 1.36	3.83 ± 1.61	t = 6.4	0.0001 *
Min. – Max.	2.0 – 9.0	2.0 – 7.0		
1 Year postoperative				
Mean ± SD.	6.08 ± 1.36	3.83 ± 1.61	t =6.3	0.0001 *
Min. – Max.	2.0-9.0	2.0 – 7.0		

*Statistically significant relation

VAS-score of overall satisfaction of thoracic sympathectomy, in group A: 77.7 % of patients were satisfied, compared to 88.8 % of patients included in group B. There was no statistically significant difference between both groups. However; patients in group B, showed more satisfaction.

The VAS-score for overall satisfaction from the surgery was 8 ± 0.82 in group A, while it was 8.75 ± 1.18 in group B patients measured by VAS-score. As for the overall satisfaction in all patients included in both study groups collectively was of statistically significant value (p=0.003).

Discussion

Primary palmar hyperhidrosis affects about 2-3% of the population. **(Wörle et al., 2007)** It causes embarrassment and problems when holding things or shaking hands. Primary palmar hyperhidrosis may be associated with plantar hyperhidrosis. Thoracoscopic sympathectomy provides a safe, permanent and rapid cure of this excessive sweating problem. Our study involved 72 patients with a main complaint of primary palmar hyperhidrosis associated with planter hyperhidrosis. The seventy-two patients have been divided into groups. None of the patients had tried any medical treatment before. The two groups were statistically matched regarding sex and age, as group A (R2-3) had a mean age of 17±5.91 years, 52.8% were females. Group B (R2-4) had a mean age of 19.72 ± 5.77 years, 66.7% were males, with the percent of males included in both groups was 56.9 %. There was a slight increase in percentage of males than that which was reported according to **Cerfolio et al., (2013).** This slight increase in the incidence of PH in this study was attributed to that the majority of patients sought medical advice for the reason of their manual work and not for social reasons and most of them were workers or students of male gender who are involved mainly in manual work.

Abu Arab and Elhamami (2021). proved that the patients' age at their study ranged from 12 to 32 years with a mean of 20.7% ± 4.7 years, which is going with our study where, group A patients had a mean of age of 17±5.91years and patients included in group B had a mean of age 19.72 ± 5.77 years, with the range of in both groups was between 9 and 35 years. **Haider and Solish (2005).** proved that palmar hyperhidrosis started early at the age of thirteen.

In this study, the operative time in group A ranged from 15 to 30 minutes; 21.58± 3.47 minutes for both sides while in group B; it ranged from 18 to 35 minutes; 22.64± 4.05 minutes. There was no statistically significant difference between both groups. In comparison to **Guirguis et al., (2023)** that stated that the duration of surgery in patients who had R2,3 sympathectomy ranged between 23 and 40 minutes with a mean of 32.75±3.12 minutes while in those who had R2-4 sympathectomy, it ranged from 39 to51 minutes with a mean of 44.53±4.02 minutes. The difference in duration between the two groups is explained by the time consumed for cauterization at in the second group. Our study showed shorter operative time than that study, which is explained by higher performance and skills of the surgeons performed the surgeries. More skilled senior surgeons.

Regarding the intra-operative complications, one patient in each group (2.8%) had an intraoperative bleeding that was controlled intra-operatively by compression and cautery. One patient in group B (2.8%) was complicated by unilateral air leak intra-operative which was managed by insertion of ICT connected to under water-seal. **Abu Arab and Elhamami (2021).** stated in their study that intra-operative bleeding occurred at 1.3% of the patients their study showed lower incidence of intra-operative bleeding which is justified by that the senior surgeons performed the procedure. Meanwhile, ICT insertion for pneumothorax was of lower incidence at

our study which is justified by limited number of patients included. Regarding the post-operative complications, our study showed one patient with superficial wound infection (2.8%) in group A. Which was managed by frequent dressing with betadine and bacitracin spray. **Abu Arab and Elhamami (2021)** stated that (2.7%) of the patients in their study showed superficial wound infection which also managed by daily dressing. While, **Neumayer C et al., (2005)** stated that (0.7%) of the patients had wound infection (0.7%) was locally treated and required no systemic antibiotic therapy.

As regard CH, twelve patients in group A; showed mild CH (33.3%), two patients (5.6%) showed moderate CH and no patient showed severe degree of CH, with total 14 patients suffering from CH (38.9%). While in group B; fourteen patients (38.9%) showed mild CH, four patients (11.1%) showed moderate CH and; no patient; too; showed a severe form of CH.

The degree of CH experienced by patients; measured by VAS-score; in group A was 1.06 ± 1.53 while it was 1.42±1.69 in group B patients. In comparison to the results of the study published by **Wei et al., (2021)** It was noted that CH occurred in 37.6% of R3 sympathectomy level patients and in around 47.7% of R4 sympathectomy level patients. This result is inconsistent with the most of the published results in literature, suggesting that R3-4 had more effect on PH treatment with low possibility and low incidence of incidence of postoperative CH**.(Liu et al., 2009; Wolosker et al., 2008)** Some other authors reported that the less levels of interruption to sympathetic chain will lead to lesser incidence and lesser severity of CH; however; others reported that it has no effect on CH.**(Licht and Pilegaard, 2004; Yano et al., 2005)**

Comparing to **Abu Arab and Elhamami (2021).** proved that the VAS-score for CH 2.77±1.004, while in our study the VAS-score in group A was 1.06 ± 1.53 and in group B; the VAS-score was 1.42±1.69. This was justified by their high number of patients included in their study.

In this study, the VAS-score for the degree of PH was assessed preoperatively and postoperatively. Pre-operatively, the mean of the VAS-score in group A was found to be (8.61 ± 1.20), while in group B; it was (8.61 ± 1.05). Post-operatively, the VAS-score in the two groups was followed up after one week, six months and one year.

In group A patients, the mean of post-operative VAS-score for PH at one week was 1.28 ± 0.45. It was the same even after six months and at one year follow-up after surgery. While in group B, the mean of the VAS-score after one week of surgery was 1.44 ± 0.56, and it remains approximately at the same value after six months. While at one year following the surgery the mean VAS-score of 1.39 ± 0.49. There was a change regarding the postoperative VAS-score of the degree of PH in patients of group B at six months and one year as two patients (5.55%) changed their VAS-score where they showed more improvement after 6 months and showed a higher level of satisfaction.

In comparison with **Abu Arab and Elhamami (2021).** where VATS sympathectomy was done to levels R2-R4, they used the VAS-score for the degree of PH pre- and post-operative, where post-operative VAS -score were taken after one week, one month and six months. They showed pre-operative VAS-score of 9.84 ± 0.5, and the postoperative VAS-score at one week, one month and six months were 0.32 ± 0.47, 0.095 ± 0.29 and 0.095 ± 0.29 respectively. There was statistically significant difference between pre- and post-operative VAS-score (p=0.0001). In their study, all patients showed significant cure and improvement from PH. Their study goes with our study where all patients included in our study had complete satisfaction and cure from PH with significance improvement between pre- and post-operative VAS-score.

However, in their study, they showed less VAS-score in the post- operative range due to the higher number of patients included in that study group than that in our study.

Other studies applied interruption of the sympathetic chain at different levels to get better effect. **Neumayer et al. (2005)** had reported an approximate success rate of 80% for treatment of PH when he interrupted the sympathetic chain at the level of the fourth ganglion. While **Martínez et al. (2015)** studied the effect of the fourth to fifth ganglion during VATS sympathectomy for treatment of PH and he reported 100% success rate. On the other hand, **Miller and Force (2007)** showed 99% success rate with interruption of the second thoracic ganglion for PH, all of them goes with our study for the success rate for PH.

In our study, PLH improvement following surgery in group B; showed a high statistically significant value (p= 0.002) with a higher prevalence of PLH improvement in such patients. Twenty-six patients (72.2%in group B and two patients in group A (5.6%) showed improvement in associated PLH, this was justified that the interruption of R4 and/or the multi-level interruption from R2-R4 had a great impact and direct link to the PLH improvement. The pre-operative VAS-score for PLH was assessed. The mean of VAS-score in group A patients was 6.11 ± 1.14, while in group B; it was 5.97 ± 0.88. There was no significant statistical difference between both studied groups regarding the pre-operative VAS-score for plantar hyperhidrosis.

While Post-operatively, the VAS-score regarding the degree of PLH in the patients of the two groups was followed up after one week, six months and twelve months. In group A, there was no change during the follow up at the three intervals with where the mean of the measured VAS score was 6.08 ± 1.36, while in group B, the mean of post-operative VAS score was 3.83 ± 1.61 and also; it showed no change at the three follow up intervals. It was noted that there was a high statistically significant difference between both means of the VAS -score of both regarding the improvement of PLH in group B (p =0.0001).

Our study goes with the results published by **Abu Arab and Elhamami. (2021).** who stated that VATS sympathectomy was done at thoracic levels (R2-4), was associated with an improvement of PLH in 79.7% of patients. Their study assisted the degree of PLH pre- and post-operatively at three different time intervals on VAS-score. The degree of PLH pre-operatively was 9.52±0.747 and post-operatively; the VAS -score showed improvement without significant changes in the 3-time intervals with mean score of 4.575±0.497 (p=0.0031*).

Wolosker et al., (2013) stated that the patients who underwent VATS sympathectomy at the R2 and R4 levels separately, showed improvement of PLH; during the follow-up intervals at one week, six months and one year. The improvement percentage started with 50% and declined to 23.4% at the end of the follow-up at one year. However, those patients who underwent sympathectomy at R3 level did not show any improvement in PLH.

Miller and Force (2007) showed that 64% of patients had an improvement in the PLH, following by VATS sympathectomy at R2. While **Neumayer et al., (2005)** stated that 42.4% of the patients who made VATS sympathectomy at 4th thoracic level, showed an improvement in PLH; there was strong direct effect in group B patients, where an interruption of more levels; reaching R4; showed an improvement of PLH than in patients in group A. This may be explained by the fact that the thermal effect may extend beyond and distal to R4 level. While, in those two patients who showed improvement in group A, the improvement may be due to decrease in the social stresses after complete dryness of the palms which may slightly improve the degree of PLH. Still, we need much more studies on different

level of pathophysiology and anatomy of the sympathetic chain.

Conclusion

Video-assisted thoracoscopic sympathectomy is the treatment of choice for a complete cure of PH. There is a strong relation between VATS sympathectomy at the R2 to R4 levels and the improvement of PLH. There was a strong positive correlation between the number of thoracic level interruptions and CH.

Conflict of interest statement: none to declare.

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Ethical Considerations: We followed the declaration of Helsinki regarding studies on human subjects. Approval of the Alexandria University Faculty of Medicine Institutional Review Board (IRB) and the medical ethics committee in Alexandria University was obtained. Patients were consented for operation and publishing of clinical data.

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Author contribution: Authors contributed equally in concepualization. A.M.E, W.S.A, and A.M.R. operated cases with other staff of the departement.M.M.Z. revised the study methodology and the manuscript. W.S.A., A.M.E. revised statistical analysis. Writing and revision of the manuscript was done by A.M.E, W.S.A., and M.M.Z

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