Evaluation of interleukin-6 (IL-6) estimation in autopsied chest traumatic cases: Prospective study

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ABSTRACT

Objectives: To find out if interleukin 6 (IL-6) can be considered as a traumatic death marker or not (traumatic or non-traumatic). **Background:** Trauma is considered as one of public health problems worldwide causing high morbidity and mortality in developed and developing areas in the worlds. A chest trauma is any state of physical force causing injury to the chest including the ribs, heart and lungs. Cytokines are regarded as important mediators in the pathological process of inflammation. In addition, chest trauma or trauma in general has no biological marker. Role of cytokines and its relation with trauma and injuries have not been researched satisfactorily. **Subject, material and methods:** one hundred cadavers with chest trauma sent to Zeinhom governmental mortuary as well as non-traumatic dead cases died in Menoufia university hospital and blood samples tested for interleukin 6 level by interleukin – 6 ELIZA (enzyme linked immune-sorbent assay) kits, cases divided into group I (control healthy cases), group II (that divided into two subgroups; subgroup II a include non-traumatic dead cases except cases died from myocardial infarction (MI), subgroup II b includes non-traumatic cases (died from MI) and group III (traumatic chest injuries deaths). **Results:** There is a highly significant difference between non-traumatic dead cases (not including MI cases) II a subgroup and traumatic dead cases III (P value < 0.001). As regarding to the mean of interleukin level it is higher in group III (58.3±20.6), it is above 3.5 folds as its value in non-traumatic dead cases (not including MI cases) II a subgroup (15.7±2.78). There is a highly significant difference between non-traumatic MI dead cases II b subgroup and traumatic dead cases III. As regarding to the mean of interleukin level, it is higher in traumatic dead cases group III (58.3±20.6) as it is above 3.5 folds as its value in non-traumatic MI dead cases II b subgroup (15.3±2.61). **Conclusion:** it is revealed that definitely IL6 is closely associated and increased with traumatic injury and can be considered as a trauma biomarker.

Keywords; chest, trauma, interleukin-6, autopsied, penetrating, non-penetrating, traumatic death.

INTRODUCTION

Trauma is considered as one of public health problems worldwide causing high morbidity and mortality in developed and developing areas in the worlds **(Park, 2000; Lema et al., 2011)**.

Injury is discontinuity of body tissue due to an external force either intentional or accidental, resulting from a sudden exposure to energy (mechanics, electricity, heat, chemicals or radiation) generated through body and instrument interaction **(Krug, 1999 and Gezairy, 2009)**.

Globally, 10% of all trauma admissions results from chest injuries and 25% of trauma-related deaths are attributable to chest injuries **(Miller and Mansour, 2007).**

A chest injury (chest trauma) is any state of physical force causing trauma to the chest including the ribs, heart and lungs **(Andrew et al., 2002).**

Physician uses different trauma scores to identify severe thoracic injury, and in case of chest trauma uses its specific score (thoracic trauma severity score). Severe thoracic trauma considered as a major cause of death. **(Martinez et al., 2016; Moon et al., 2017).**

Chest injuries can be caused by blunt instruments as in cases of road traffic accident, falling from a height or assault. Also chest injuries can be caused by penetrating traumatic instruments such as stabbings or puncture wounds including firearm injuries cases **(Feliciano et al., 2012).**

In physical assault, the nature of injury depends on the weapon and how the weapon used to commit the injury, nature of the affected tissue and the performance of the applied force (for example, if it is completely transmitted to the tissue or part lost in case of moving the body) **(Ahmed et al., 2012).**

The mechanical injuries generally could be classified into two main types depending on the causative instrument as:

∗ Blunt injuries as abrasions, contusions, contused wounds, puncture and firearm wounds (non-penetrating and penetrating).

* Sharp injuries as cut and stab wounds (non-penetrating and penetrating) **(Milisavljević et al., 2012)**.

There may be different types of skin injuries to the body that could be localized or distributed all over the body. Absence of external local signs of injury to the skin does not exclude presence of serious affection of the internal organs of different body parts by traumatic force **(Sharma et al., 2011).**

Cytokines are regarded as important mediators in the pathological process of inflammation **(Giannoudis et al., 2004; Okeny et al., 2015; Thomas, 2015)**.

Interleukin 6 (IL-6) is one of cytokines secreted by leukocytes. It is secreted in the body whenever inflammation occurs and it is responsible for fever in infectious and non- infectious (autoimmune) cases. When it is secreted in case of inflammation or injury, it results in synthesis of different inflammatory process elements, responsible for the body defense and at the same time can exaggerate the illness **(Rincon, 2012; Thomas, 2015; Stanken and Poschenrieder, 2015)**.

Role of cytokines and its relation with trauma and injuries have not been researched satisfactorily. As in case of blunt chest injuries, there are no sufficient available vivo data upon their relation with cytokines release. In addition, we do not know any chest trauma biological marker **(Strecker et al., 2002)**.

AIM OF THE WORK

As it is postulated that interleukin-6 (IL-6) increases in traumatic cases, IL-6 has been investigated to find out if it can be considered as a traumatic death marker or not and if it can point to the cause of death (either traumatic or non-traumatic).

SUBJECTS, MATERIAL AND METHODS

Subjects:

- The study performed on one hundred cadavers with chest trauma sent to Zeinhom governmental mortuary as well as non-traumatic dead cases picked from cases died in Menoufia university hospital and healthy control cases (after excluding the exclusion criteria).

» Cases divided into 3 main groups as follows:

*Group I: control healthy cases (10 cases) for interleukin – 6 estimation.

*Group II: non-traumatic deaths for interleukin – 6 estimation and it is further divided into two subgroups as follow:

- Subgroup IIa: representing non-traumatic dead cases except cases died from myocardial infarction (MI) (10 cases).

- Subgroup II b: representing non-traumatic cases died from myocardial infarction (MI)(10 cases).

*Group III: traumatic chest injuries dead cadavers (100 cadavers) for pattern and interleukin – 6 estimation.

* Material:

- A sheet for chest trauma autopsied cases' findings was designed.

- Blood sampling syringes, plain (non-preservative) tubes and storage ice device (ice box).

- Centrifuge equipment.

- Freezer – 80 C in central laboratory in faculty of medicine – Menoufia University (M.U.) for storage of samples after being centrifuged and serum kept in clean plastic plain tubes.

- Interleukin – 6 ELIZA (enzyme linked immune-sorbent assay) kits.

- Equipment needed for samples analysis steps by ELIZA in central laboratory in faculty of medicine, Menoufia University (M.U.).

* Methods:

- After a valid informed consent (After explaining the aim and methods of the work) was taken from each case involved in the study as control cases, from Zeinhom governmental mortuary authority, from Menoufia university hospital and heirs of deceased and from ethics committee in Menoufia university - faculty of medicine and the approval was taken.

- The consent form was a design from postgraduate and research unit (in faculty of medicine, Menoufia University)

- A sheet for chest trauma autopsied cases' findings was fulfilled.

  ❖ Exclusion criteria: it includes (Infection, Inflammatory diseases as autoimmune disease, Pregnancy and Malignancy).

  ❖ Inclusion criteria: after excluding the exclusion criteria the venous samples (distal veins from the heart are more preferred) should be withdrawn in the first 24 hr. after death to exclude errors happen due to post-mortem changes especially putrefaction.

- ELIZA method used for detecting interleukin – 6 (**Mimasaka et al., 2007).**

- By using ELIZA kits for interleukin – 6 (IL-6), samples (after being melted) were tested and the results were collected and printed (normal blood range of interleukin-6 = 5-15 ng/L) **(Mimasaka et al., 2007)**.

- All data were sent for **statistical analysis** as follows:

Data were collected, tabulated, statistically analyzed using an IBM personal computer

b- **Analytical statistics:** used to find out the possible association between studied factors and the targeted disease. The used tests of significance included:

Kruskal wallis Test.

Mann-Whitney test (nonparametric test): is a test of significance used for comparison

with Statistical Package of Social Science (SPSS) version 22 **(SPSS, Inc, Chicago, Illinois, USA)** where the following statistics were applied:

a- **Descriptive statistics**: in which quantitative data were presented in the form of mean ($\overline{X}$), standard deviation (SD), range, and qualitative data were presented in the form numbers and percentages.

between two groups not normally distributed having quantitative variables.

P value of >0.05 was considered statistically non-significant.

P value of <0.05 was considered statistically significant.

P value of <0.001 was considered statistically highly significant

RESULT

Figure (1) is a column chart that shows the distribution of different mode of death among the dead cases; assault by firearm weapons represents 44% of the cases, assault by sharp instruments represents 37% of cases then assault by bunt instruments, assault by falling from a height and lastly assault by road traffic accident represents 12%, 4% and 3% respectively. Figure (2) is a column chart that shows variation pattern of local signs of chest injuries during external examination of dead cases; firearm injury took the first place as represents 44% of the cases next to it came stab wounds (37%), then contusions and contused wounds that represent (19%) of cases and lastly cut wounds that represent the least one (4%).

Figure (3) pie chart that shows traumatic dead cases distribution regarding body regions trauma illustrated that chest trauma accompanied with other body region trauma was (56%), while isolated chest trauma represented (44%) of traumatic dead cases.Figure(4): is a column chart that

shows the distribution of the associated different body

regions trauma among studied chest trauma dead cases; where abdomen had the highest percentage (43%), followed by head trauma (40%) then upper limbs (29%) and lastly lower limbs (23%). Figure (5): pie chart that shows distribution of studied chest trauma dead cases according to the instrumental application on the chest where penetrating instruments (either sharp or blunt) represents most of cases (80%) then non-penetrating blunt one represents (20%). Thoracic wall contusion from inner aspect at level of 2nd and 3rd left ribs and the left hemothorax in case of polytrauma (assault by blunt non-penetrating instrument) as in photographic picture (1). Cases presented with stab in anterior thoracic wall on left side (start of stab wound tract under the level of left 4th rib after dissection of the skin), caused by sharp penetrating instrument as shown in photographic picture (2). Cases of firearm gunshot had multiple small rounded shots entry wounds,

assault at left side of the thorax, caused by penetrating instrument shots as in photographic picture (3). Cases with stab chest wound on left side were with stab heart in left ventricle that was revealed during dissection of the chest (sharp penetrating instrument) (dagger) as shown in photographic picture (4).
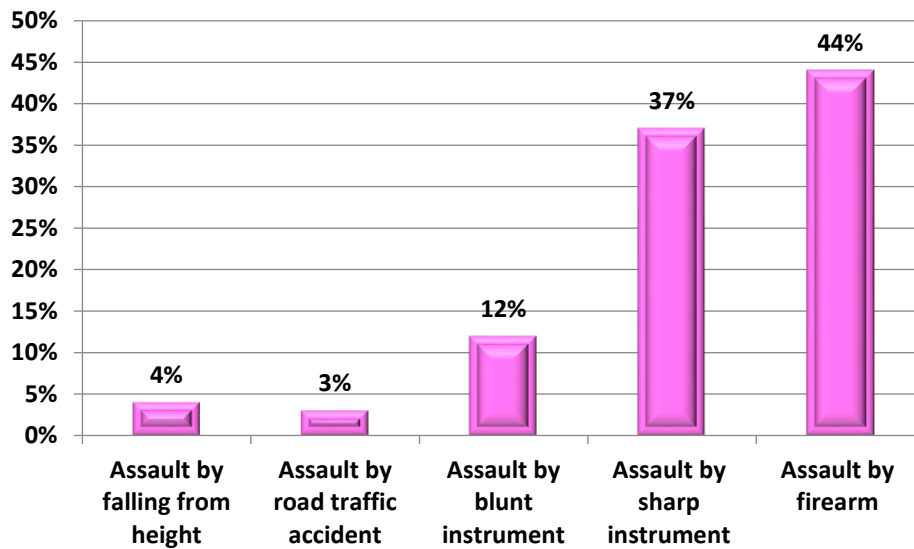

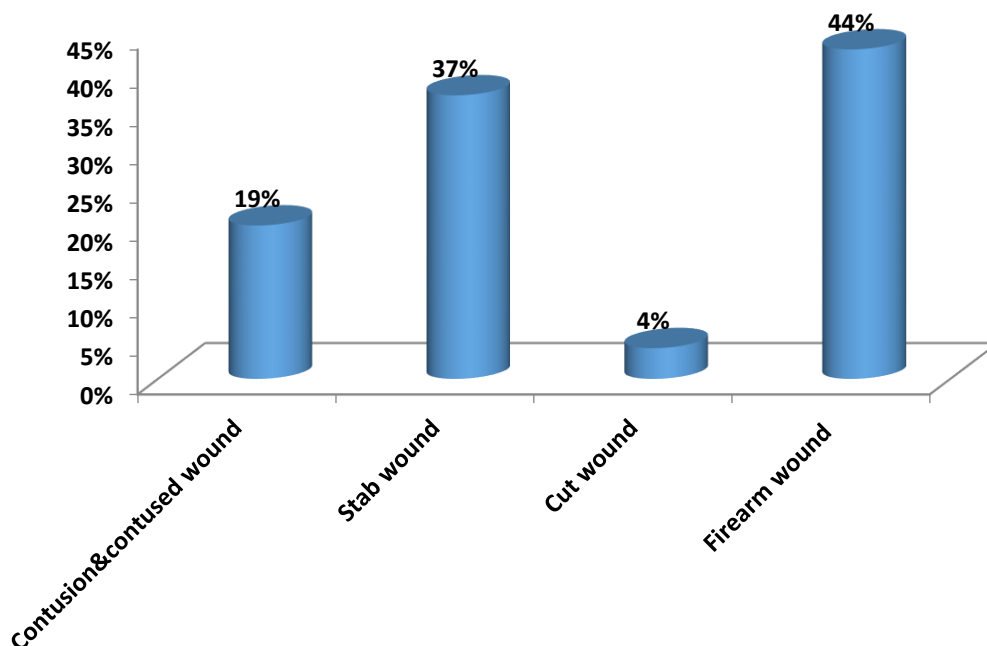**Figure (1): Distribution of different mode of death among the traumatic dead cases (No = 100).**
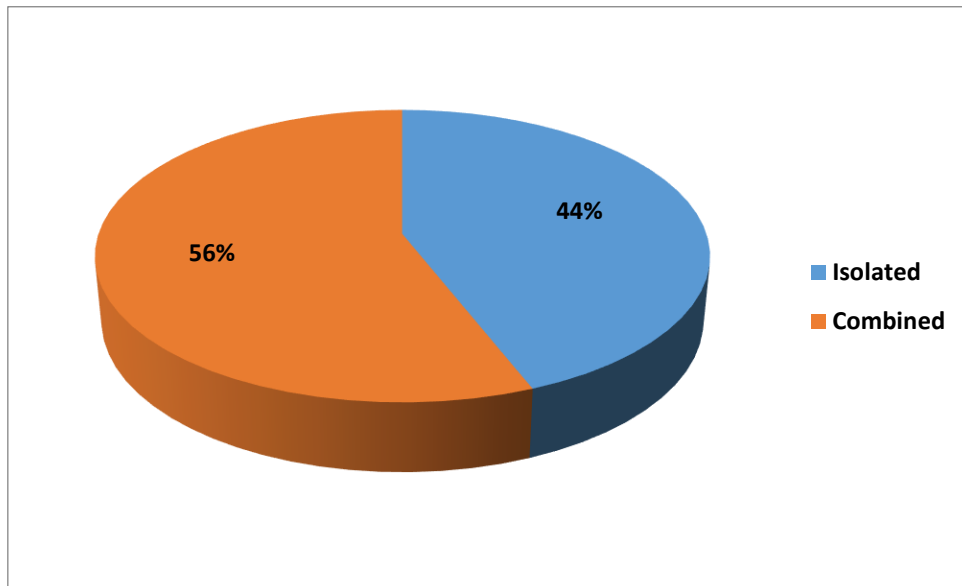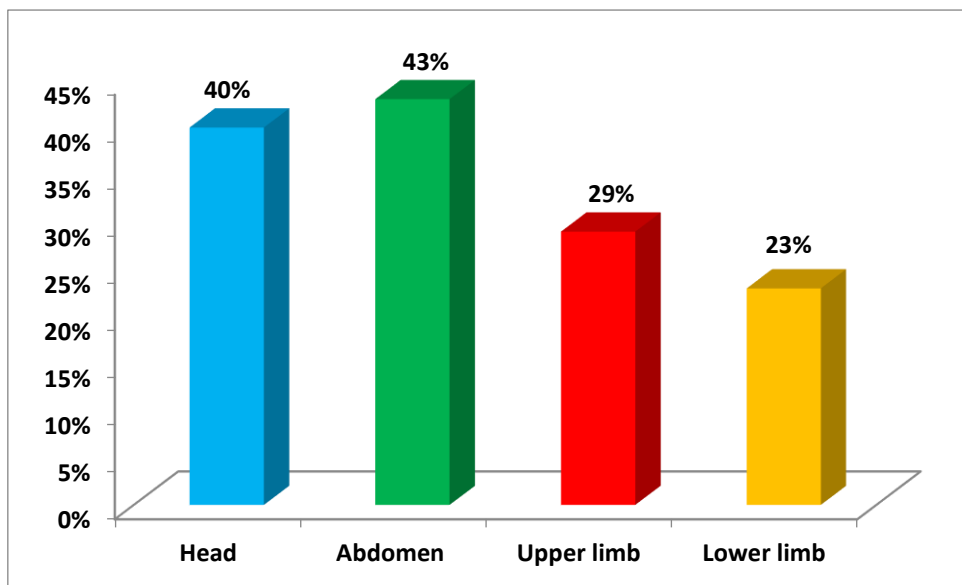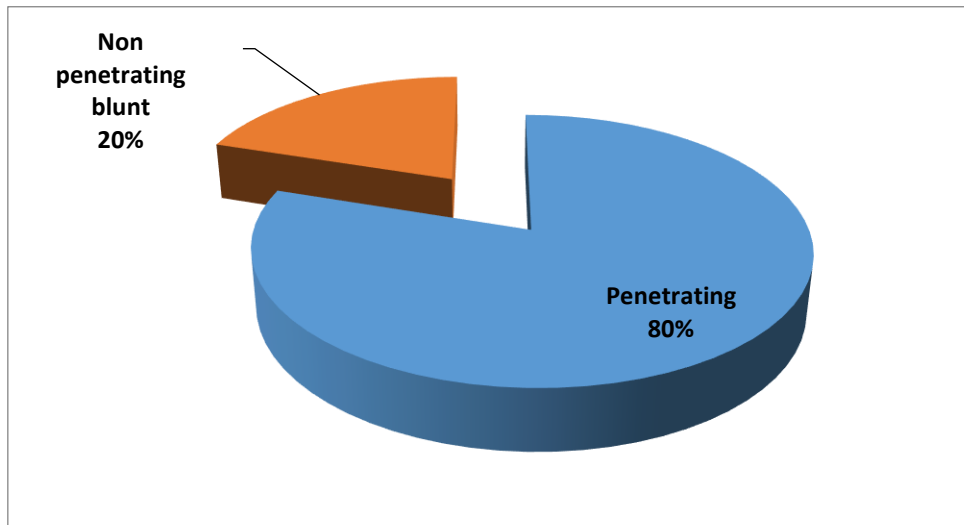

Figure (2): Pattern of local signs of chest injuries during external examination of traumatic dead cases (No = 100).

Figure (3): Distribution percentage of traumatic dead cases regarding body regions trauma (No = 100).
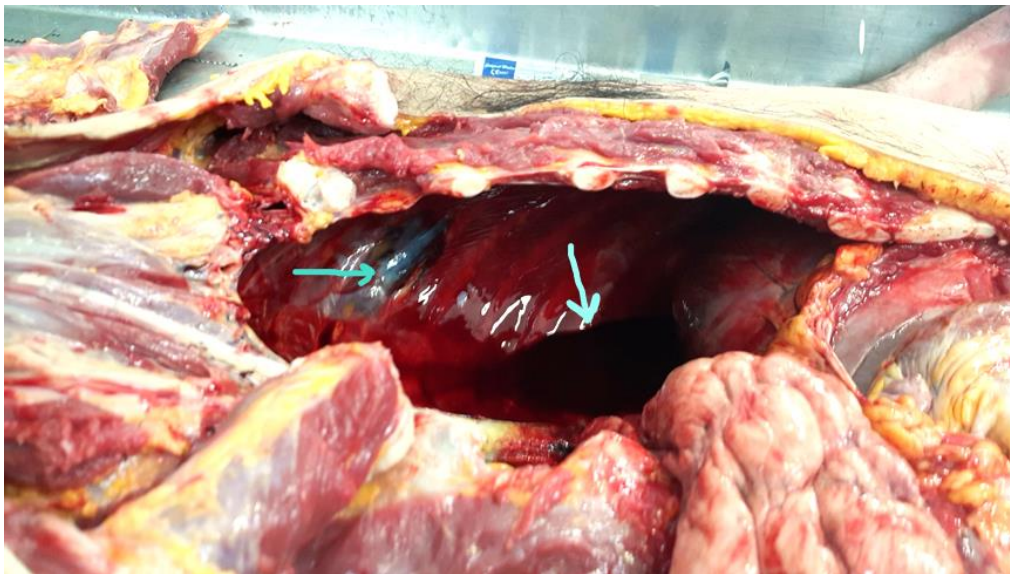

Figure (4): Distribution of the associated body region trauma among studied chest trauma dead cases (No=100).

Figure (5): Distribution of studied chest trauma dead cases according to the instrumental application on the chest (No=100).

*The study cases were varied, that included cases such as:

Photographic picture (1) shows arrow points to thoracic wall contusion from inner aspect at level of 2nd and 3rd left ribs (the left one) and another arrow (on the right side) points to the left hemothorax in case of polytrauma (assault by blunt non-penetrating instrument).
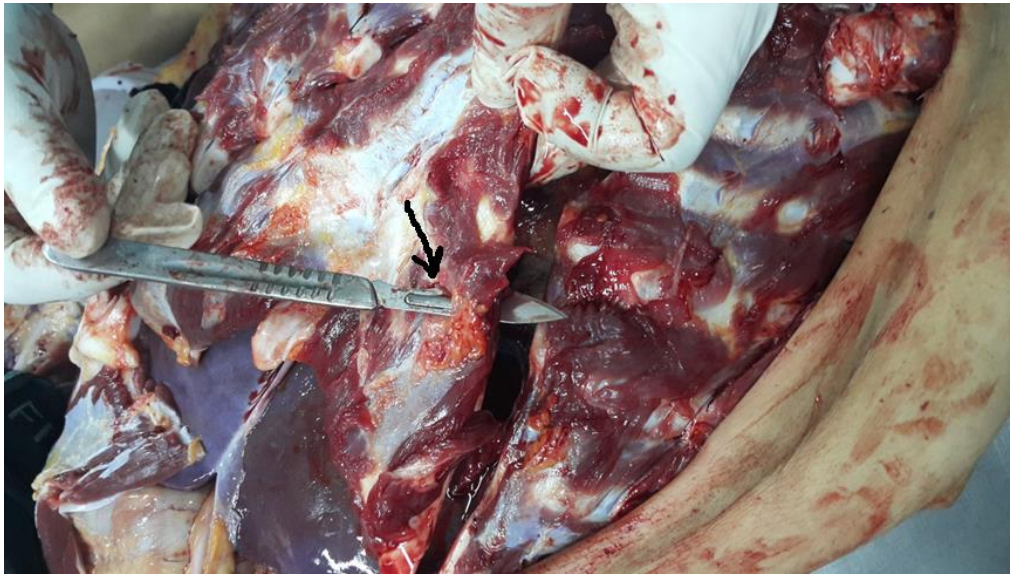
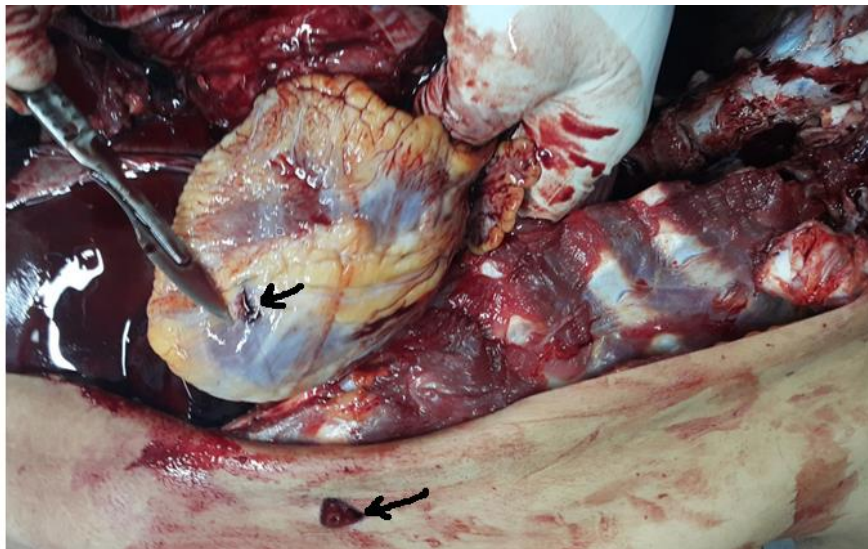**Photographic picture (2): shows arrow points to a scalpel inserted in the anterior thoracic wall on left side (start of stab wound tract under the level of left 4th rib) after dissection of the skin, caused by sharp penetrating instrument.**

Photographic picture (3): shows a case of firearm gunshot (arrow points to the multiple small rounded shots entry wounds) assault at left side of the thorax.

Photographic picture (4) shows the scalpel tip and arrow points to stab heart in left ventricle and the other arrow points to stab wound on the skin on left side of the thorax during dissection of the chest (sharp penetrating instrument) (dagger).

Table (1): Interleukin-6 (IL-6) level (ng/L) among studied groups:

Studied variables	Mean ±SD	Range	Kruskal wallis Test	Post hoc P value
Group I (No=10)(control)	7.34±1.74	4.67 – 9.78		
Group II a (No=10)(non – traumatic without MI)	15.3±2.61	11.7 – 19.5	70.9 **0.001**	**P1:0.001**** **P2:0.001**** **P3:0.001**** P4:0.705 **P5:0.001**** **P6:0.001****
Group II b (No=10)(non-traumatic with MI)	15.7±2.78	11.7 – 19.0		
Group III(No=100)(traumatic dead cases)(TD)	58.3±20.6	30.3 – 49.4		

Highlight significant

****Highly significant**

MI = myocardial infarction   No = number

P1: Comparison between group I and group II a

P2: Comparison between group I and group II b

P3: Comparison between group I and group III

P4: Comparison between group II a and group III b

P5: Comparison between group II a and group III

P6: Comparison between group II b and group III

This table shows that:

There is a highly significant difference regarding interleukin-6 (IL-6) levels between (control healthy living cases = I) and (non-traumatic dead cases not including MI cases = II a) **(P1 value < 0.001)** and regarding the mean of interleukin level it is higher in (II a subgroup) (15.3±2.61) it is above two folds as its value in (control group I) (7.34±1.74).

There is a highly significant difference regarding interleukin-6 (IL-6) levels between (control healthy living cases = I) and (non-traumatic died cases from MI = II b) **(P2 value < 0.001)** and regarding the mean of interleukin level it is higher in (II b subgroup) (15.7±2.78) it is above two folds as its value in (control group I) (7.34±1.74).

There is a highly significant difference regarding interleukin-6 (IL-6) levels between (control healthy living cases = I) and (traumatic dead cases = III) **(P3 value < 0.001)** and regarding the mean of interleukin level it is higher in (group III) (58.3±20.6) it is above eight folds as its value in (control group I) (7.34±1.74).

There is no significant difference regarding interleukin-6 (IL-6) levels between (non-traumatic dead cases not including MI cases = II a) and (non-traumatic dead MI cases = II b) **(P4 value > 0.705)** and regarding the mean of interleukin level it is nearly the same in both groups as it is (15.7±2.78) and (15.3±2.61) respectively.

There is a highly significant difference regarding interleukin-6 (IL-6) levels between (non-traumatic dead cases not including MI cases = II a subgroup) and (traumatic dead cases = III) **(P5 value < 0.001)** and regarding the mean of interleukin level it is higher in (traumatic dead cases group III) (58.3±20.6) it is above 3.5 folds as its value in (non-traumatic dead cases not including MI cases = II a subgroup) (15.7±2.78).

There is a highly significant difference regarding interleukin-6 (IL-6) levels between (non-traumatic MI dead cases II b subgroup) and (traumatic dead cases III) **(P6 value < 0.001)** and regarding the mean of interleukin level it is higher in (group III) (58.3±20.6) it is above 3.5 folds as its value in (non-traumatic MI dead cases II b subgroup) (15.3±2.61). **Table (2):** There is a highly significant difference between isolated and combined chest traumas regarding the mean interleukin levels it is higher in combined group (72.8±14.4) it is about two folds as its value in isolated subgroup (39.1±6.86).

Table (2): Relation between interleukin-6 (IL-6) levels (ng/L) and site of injury (regional affection) among studied traumatic dead cases (No = 100):

Studied variables	Site of injury (regional affection)		Mann Whitney test	P value
	Isolated (N=44)	Combined (N=56)		
Interleukin level				
Mean ± SD	39.1±6.86	72.8±14.4	8.54	**0.001****
Range	30.3 – 54.1	54.1 – 94.4		

****Highly significant**

DISCUSSION

Trauma causes devitalization to different body tissues which by its turn is responsible for several responses including production and release of different cytokines including interleukin-6 (IL-6) (keel and Trentz, 2005).

Interleukin - 6 (IL-6) is high in severely traumatized dead cases more than its level in non-traumatized dead cases and many other studies had reported that serum IL-6 levels are positively associated with severity of trauma and so death (Gebhard et al., 2000; Strecker et al., 2002; Steinsballe et al., 2009; Andruszkow et al., 2014; Okeny et al., 2015; Sousa et al., 2015).

Thomas, (2015) reported that the increased release of (IL-6) took place upon tissue injury and devitalization as a defense mechanism of body against tissue damage **(Thomas, 2015)**.

Levels of (IL-6) is more higher in cases of chest trauma accompanied with another body region injury as abdomen or head injury and this matched with study of Strecker et al., (2002) and winter et al., (2004). As the more multiple body regions injured and damaged, the more (IL-6) increased **(winter et al., 2004 and Thomas, 2015)**.

In this study penetrating instrumental traumas are significantly high representing 80% of the cases and this is agreed with by Lema et al., (2011).

On contrary study of Ludwig and Koryllos, (2017) stated that blunt instrumental thoracic injury is more common but blunt thoracic injury by itself may be non-penetrating or penetrating so this is also can be matched with the results concerning penetrating thoracic trauma in this study.

Assault pattern is also represented here by percentage of 100% of all cases and this may throw light on increasing violence in our region, trying to reach or invent preventive and protective measures by all society sectors.

This study shows firearm injury takes the first place as represented 44% of the cases and stab wounds represented 37%, then contusions and contused wounds that were 19% of cases and this is agreed with by Lema et al., 2011 study.

This is can be rationalized by the increase in global violence and crimes, also presence of weapons variety in the markets all over the world **(Ludwig and Koryllos, 2017)**.

Polytrauma (combined chest and other body region injury) cases here are representing 56% and this is agreed with by Liman et al., (2003) and they had higher interleukin - 6 (IL-6) levels (about two folds) than that in isolated chest trauma and indicating increased severity and so possibility of increased mortality in the cases and this is agreed with by Bernardin and Troquet, (2012) and Milisavljevicć et al., (2012).

This is may be due to the increased release of interleukin - 6 (IL-6) upon tissue injury and devitalization and this increase is directly proportional to the bulk of the damaged tissues that is more increased by its turn in cases of combined chest trauma than in cases of isolated chest trauma **(Thomas, 2015)**.

The distribution of the associated body regions trauma among studied chest trauma dead cases was as follows abdominal trauma represented (43%), followed by head trauma (40%) then upper limbs (29%) and lastly lower limbs (23%) and this is agreed with by Liman et al., (2003).

This can be due to the close relation between thorax and each of abdomen, head and upper limbs other than the relation between thorax and lower limbs.

CONCLUSION

From this study, it can be concluded that:

- Interleukin - 6 (IL-6) definitely is closely associated and increased with trauma and can be considered as a trauma biomarker.
- This relation can play a vital role in traumatic forensic cases, so it can be considered as an evidence of trauma sharing in trauma diagnosis like imaging and dissection.

RECOMMENDATIONS

✓ Interleukin – 6 (IL-6) in relation to living humans experiencing trauma should be further researched and its relation with trauma severity and so intervention and outcome in these patients.

✓ Nevertheless, more researches should evaluate this relation regarding time passed since injury (and so can point at crime and assault time) and time passed since death after excluding the exclusion criteria for IL6 measurement.

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تقييم قياس نسبة الإنترلوكين -6 فى الحالات الإصابية بالصدر والتى تم تشريحها: دراسة مستقبلية

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نبذة مختصرة:

مقدمة: تعتبر الإصابات مشكلة صحية عامة منتشرة علي مستوى العالم ومرتبطة إرتباطا وثيقا مع نسبة العجز و الوفيات فى الدول المتقدمة و النامية علي حد سواء. إصابات الصدر تحدث عن طريق تأثر الصدر بأى قوة ميكانيكة سواء بالضلوع أو الاعضاء الداخلية كالقلب والرئة. وتعد السيتوكاينز ومنهم الإنترلوكين-6 من المحفزات المهمة لعملية الإلتهاب بالجسم. والإصابات عموما ولا سيما الإصابات بالصدر لا يوجد لها دلالة بيولوجية في الجسم. والعلاقة بين الإصابات و السيتوكاينز لم تتم عليها الدراسات والابحاث بشكل مرضى. **الهدف من البحث:** معرفة هل يمكن إعتبار الإنترلوكين-6 من الدلالات البيولوجية علي الإصابات الصدرية وبالتالي حالات الوفيات الناتجة عن إصابات الصدر أم لا؟. **الحالات والأدوات المستخدمة وطرق البحث:** 100 حالة وفاة تم إرسالها وتشريحها بمشرحة زينهم وحالات وفاة أخرى لا إصابية من مستشفى جامعة المنوفية وتم تحليل وقياس نسبة الإنترلوكين-6 في عينات الدم التى تم سحبها من الحالات وذلك باستخدام مجموعة الإليزا وتم تقسيم الحالات إلي ثلاث مجموعات المجموعة الأولي وهي مجموعة التحكم والمجموعة الثانية وهى مقسمة مجموعتين الأولي منها وتحتوى علي الحالات اللاإصابية المتوفية دون الحالات المتوفاه نتيجة إحتشاء عضلة القلب والمجموعة الثانية منها تحتوى علي حالات الوفيات اللاإصابية المتوفية نتيجة إحتشاء عضلة القلب والمجموعة الثالثة وتحوى حالات الوفيات الناتجة عن إصابات الصدر. النتائج: وجود إختلاف كبير للغاية بين المجموعة الثالثة والمجموعة الأولى للمجموعة الثانية من الحالات حيث أن المتوسط الإحصائى لنسبة الإنترلوكين في الدم أعلي بالمجموعة الثالثة (50,3 ± 20,6) مما يجعلة أكثر من ثلاث و نصف أضعاف قيمتة بالمجموعة الأولي للمجموعة الثانية (15,7 ± 2,78) كما يوجد إختلاف كبير للغاية بين المجموعة الثالثة والموجموعة الثانية للمجموعة الثانية من الحالات حيث أن المتوسط الإحصائى لنسبة الإنترلوكين في الدم أعلي بالمجموعة الثالثة (50,3 ± 20,6) مما يجعلة أكثر من ثلاث و نصف أضعاف قيمتة بالمجموعة الثانية (15,3 ± 2,61). **الإستنتاج:** الإنترلوكين-6 يزيد في الحالات الإصابية ومن الممكن إعتبارة دلالة بيولوجية علي حدوث الإصابة.