RESULTS

RESULTS

44 pigmented male guinea pigs were included in this study. Their weight ranged from 350 to 500 gms.

In this part examples of histopathologic photos will be presented for each animal group, in a manner to compare those animals in control group, those injected by cisplatin in acute regimen and those injected in chronic regimen. Also to compare the difference between the two different method of preparation of the cochlea.

The statistical study was planned in 3 directions:

1- Comparing cochlear turns in each group regarding the severity of degenerative changes occurred.
2- Comparing the traditional method of tempoal bone embedding by decalcification and the new method which depends on MMA embedding.
3- Comparing the two main study groups together to study the odds ratio between them.

CONTROL GROUP

Subdivision A:

Examination of control group cochleas prepared by MMA: (n = 4 animals = 8 cochlear specimens)

The specimens were observed and photographed by a Zeiss Axioplan photomicroscope. All animals injected with mannitol and saline showed normal histological structure of the organ of corti, supporting cells, stria vascularis and spiral ganglion in all turns as showed in Fig. (8).

1- Examination of organ of corti:

Fig. (10) stained by H & E shows a longitudinal section in the outer hair cells which were cylinderical in shape; the upper pole of the cell carried the stereocilia, the nucleus was rich in chromatin and was found in the lower part of the cell. The inferior pole of the cell was rounded and in contact with the nerve fibers which supply the hair cell.

The inner hair cells had also normal structure which were in a single row relatively short and flask-shaped, each inner hair cell is supported and completely enveloped-except for the cuticular surface-by an inner phalangeal cell. As in outer hair cell, infracuticular, supranuclear and infranuclear regions can be observed.

The supporting cells had normal structure, the inner and outer pillar cells and Deiter's cells which form the main supporting frame work of the organ of corti. Both the inner and outer pillar cells are morphologically similar, the cell apex (head portion) and base (foot plate) are longer than the mid portion. The nucleus is located at the basal portion, also the Deiter's cells which had a lower portion reaching from the basilar membrane to the base of an outer hair cell and a slender process which

runs obliquely to another hair cell, the cells share with the pillar cells in the formation of the reticular membrane.

The border cells, inner phalangeal cells, cells of Hensen, claudius cells and Boettcher cells were in normal structure and arrangement.

2- Examination of stria vascularis:

Also Fig. (12, 14) which stained by H & E and Masson stains showed normal histological structure of stria vascularis, the stria appears as very dense tissue containing a network of capillaries which run mainly in the longitudinal spiral direction. The border of the individual cells can hardly be distinguished. There are three different types of cells in the stria namely marginal, intermediate and basal cells. The marginal cells form the lateral margin of the cochlear duct and are the only cells in the stria that in contact with endolymph. Other cells without infolding, intermediate cells, do not reach the endolymph surface. The basal cells are flat and large, and seen to form a very effective diffusion barrier against the spiral ligament. There are tight junctions between the marginal cells and between the basal cells.

3- Examination of the spiral ganglion:

The spiral ganglion in the modiolus on the inner wall of the spiral lamina was also in normal structure, the cells are bipolar with one process projecting to the hair cells. (N.B.: the other process projects towards the cochlear nucleus in the brain stem in a live animal). The axon showed normal myeline sheath without disintegration as in Fig. (16) which stained with H & E, and silver stains.

4- Examination of spiral ligament:

The spiral ligament and limbus spiralis appeared normal without changes as in (Fig. 12,14).

Subdivision B:

Examination of the cochlea of the control animals prepared by Decalcification method: (n = 4 animals = 8 cochlear specimens)

The specimens were stained also by previous staining as mentioned before and light microscopic fotos were presented for the organ of corti as in Fig. (11) and for stria vascularis which stained by H & E and Masson as in Fig. (13, 15), respectively.

Also Fig. (17) showed the spiral ganglion which stained by H & E and silver stain, we observed from these fotos that there were no histologic changes in all turns but there were a great difference in clearance, maintaining bone integrity for the specimens and fine details of the soft tissue Architecture if we compare these fotos (9,11,13, 15,17) with those specimen which prepared by M.M.A., embedding method as shown in fotos (8,10,12, 14, 16) which preserve the fine details of the cells and bone integrity. This difference of interpretation was found also in all comparison during study of the medicated groups either in acute or chronic regimen.

Fig. (8): Photomicrograph of the temporal bone after embedding in methyl-methacrylate.
A) Bone and membranous elements are well preserved.
B) Organ of corti shows excellent preservation of hair cells, supporting cells and tectorial membrane (H & E X 400).

Fig. (9): Photomicrograph of the temporal bone after decalcification and parraffin embedding
A) Bone and membranous elements are not well preserved.
B) Organ of corti did not show excellent preservation of hair cells, supporting cells and tectorial membrane.

(H & E X 400)

Fig. (10): Photomicrograph of the cochlea of the control group prepared by (MMA) showing normal outer cells, (1) inner hair cell, (2) supporting cells, (3) limbus, (4), tunnel and rods of corti (T), Basilar membrane (B) Spiral ganglion cell (5).

(H & E X 400)

Fig. (11): Photomicrograph of the cochlea of the control group prepared by Decalcification. Showing OHC (1) YHC (2) Supporting cells (3) Caludius cells (4), rods and tunnel of cort (T) Basilar membrane (B).
(H & E X 400)

Fig. (12): Photomicrograph of the cochlea of the control group prepared by (MMA) showing stria vascularis (S.V) and spiral ligament (S.L).
(H & E X 400)

Fig. (13): Photomicrograph of the cochlea of control group prepared by decalcification showing stria vascularis (S.V) and spiral ligament (S.L).
(H & E X 400)

Fig. (14): Photomicrograph of the cochlea of the control group prepared by (MMA) showing stria vascularis (S.V) and spiral ligament (S.L)
(Masson stain X 400)

Fig. (15): Photomicrograph of the cochlea of the control group prepared by decalcification showing stria vascularis (S.V) and spiral ligament(SL) (Masson stain X 400).

Fig. (16): Photomicrograph of the cochlea of the control group prepared by MMA showing normal spiral ganglion cells (H & E X 400)

Fig. (17): Photomicrograph of the cochlea of the control group prepared by decalcification showing normal spiral ganglion cells (H & E X 400).

STUDY GROUPS

The animals were divided into two main study groups; acute and chronic treated groups:

ACUTE TREATED GROUP (GROUP I)

Subdivision (A): (n = 9 animals = 18 cochlear specimens)

Examination of the cochlea which prepared by methyl methacrylate embedding method:

The pathological changes in the cochlea could be classified as 3 categories:

a- Organ of corti affection: (we used H & E for its study).

b- Stria vascularis affection: (we used H & E and Masson stains).

c- Spiral ganglion cells affection: (we used silver stain and H &E stain for its study).

A- ORGAN OF CORTI AFFECTION

Morphological changes in the organ of corti were of similar degree in quantitative pattern of hair cell degeneration and loss in all animals of the acute study group. The overall toxic affection of cisplatin was prominent in the first (basal) turn. Damage of different degrees was also seen in the second and third turns; so we noticed normal cells; degenerating cells and completely degenerated cells were scattered allover the cochlear turns as in Fig. (18, 20).

1- Outer hair cells affection:

It was the most striking change by far was seen in the organ of corti especially in basal turn lateral to the tunnel of corti; that the outer most row was slightly more affected, but degenerative process extended to a variable degree in the second and third turn. This pattern of distribution discussed statistically through table 1 and 2. Which demonstrated that there were statistical significant difference between new method and old one in revealing fine changes that occurred for OHCs at basal and middle turns (P-value < 0.05). also there was a highly statistical significant difference between 1st, 2nd and 3rd turns regarding OHCs changes (P < 0.001); and the 1st turn was highly affected followed by 2nd and then 3rd (95%, 83% and 11.1%) respectively from examined cochleas in group I and the most severe changes was also restricted for 1st turn = 72.2%.

As in Fig. (18,20) the nuclei began to become piknotic vacuolated and fragmented with a cellular death type apoptosis resulting in outer hair cell loss which involved essentially the basal turn which corresponded to the high frequency area; followed in a descending fashion by a second and third turns of the cochlea and the least apparent changes were present in the apical turn. Moreover, the outmost row was slightly more affected than the other 2 rows, the remaining outer hair cells showed missing hairs and other degenerative features.

It was founded that vacuoles appeared in the cytoplasm near the base and near the sides, it is the main pathological changes, these vacuoles could be seen also filled the cytoplasm and become very near to the cell membrane and about to rupture the plasmic membrane.

The disappeared outer hair cells were replaced by the proliferating phalangeal processes of Deiter's cells and Hensen's cells.

I noticed in one animal of the acute study group that the supporting elements were destroyed including the pillar cells, Deiter's and Hensens cells which were replaced by a layer of non differentiated cuboid epithelium resting on the basilar membrane (Fig. 21).

2- Inner hair cells affection:

Scattered minimal degree of inner hair cell degeneration was seen in the basal turns and middle turns this pattern of distribution discussed statistically through tables (3,4). Which demonstrated the same statistical finding in table (1) but (P-value < 0.001) for 1st and middle turn. Also table 4 revealed that there were highly statistical significant difference between 1st, 2nd and 3rd turns regarding IHCs changes (P < 0.0001), and 1st turn was highly affected followed by 2nd turn (72.2%, 27.8%) respectively from examined cochleas of group I the 3rd turns showed no degenerative changes for IHCs in all examined cochleas of group I. These changes were demonstrated in the following minimal changes: The whole size of the inner hair cell was decreased and the cell had irregular outline, also there were scattered missing of hairs. The ratio between the size of the cytoplasm and the size of the nucleus decreased. I noticed also that the cytoplasm appeared relatively more granular. Also vacuolization were occasionally be noted at the base of IHCs. I noticed that the varying degree of IHC degeneration which was ranging from only minor atrophy to loss of IHCs and inner pillar cells was correlated and directly proportionated to the degree of OHCs loss as in Fig. (18, 20).

3- Supporting cells affection:

The Hensen's cells and Deiter's cells were markedly swollen and in some sections filling up more or less the entire space of Nuel occurred. Alteration in the outline of the organ of corti was found in most affected specimens. These structural changes were mostly seen in the first turn but similar conditions could also occasionally be observed in the second turns, and third turn as in Fig. (18), (20) and this pattern of distribution discussed statistically through tables (5,6). Which revealed significant difference between new and old methods for interpretation of fine changes of supporting cells at 1^{st}, 2^{nd}, and 3^{rd} turns P-values were (< 0.05, < 0.05, < 0.001) respectively. Also table 6 presented highly significant difference between 1^{st}, 2^{nd} and 3^{rd} turns regarding supporting cells affection ($P < 0.0001$), and the 1^{st} and 2^{nd} turns were highly affected followed by 3^{rd} turn (88.9%, 88.9%, 11.1%) respectively from examined cochleas of group I.

It was impossible to separate the previous Dieters' and Hensen's cells from each other, even the claudius cells appeared swollen.

B- STRIA VASCULARIS AFFECTION

Study of the structure of the stria-vascularis indicated that damage was non homogeneously distributed in stria vascularis; in the same stria; sites of damage were observed adjacent to normal appearing tissue. The severe degenerated sites appeared as translucent sites. The affection was more pronounced in basal turns, middle turns in a less degree and the apical turns were the least; as it was revealed in tables (7,8). Which revealed highly significant difference between new and old methods for interpretation of degenerative changes of stria vascularis at 2^{nd} turns ($P < 0.0001$) but the difference was statistically insignificant for 1^{st} and 3^{rd} turns demonstration ($P > 0.05$). Also table 8 revealed highly significant difference between 1^{st}, 2^{nd} and 3^{rd} turns regarding stria vascularis affection ($P < 0.0001$) and the most severe affection was restricted for basal turn (27.8%) of examined cochleas in group I. But the middle turns which were affected with mild and moderate changes only (38.9%, 27.8%) respectively.

The degenerative changes appeared in the following pattern:

- Vacuolization in marginal, intermediate and basal cells.
- The nuclei had shape deformity in the form of lobulation, convolution and fragmentation severe lesions began from marginal cells and descended to basal cells as Fig. (22).

In some sections a few marginal cells in the stria vascularis were swollen and bulged into the endolymphatic space, and some cells ruptured in the endolymphatic space as in Fig. (23).

C- SPIRAL GANGLION AFFECTION

The nerve endings seemed less affected by cisplatin than hair cells, spiral ganglion cells had significantly diminished in the modiolus adjacent to the basal turns of the cochleas vs. the other portions as it was revealed in tables (9,10) which revealed statistical significant difference between new and old methods of temporal bone embedding in prediction of fine changes in spiral ganglion cells and nerve fibers at basal and middle turns (P-value < 0.001 and < 0.05) respectively. Also table 10 presented a highly statistical significant difference between 1st, 2nd and 3rd turns regarding the spiral ganglion affection (P-value < 0.0001) and basal turns of 10 cochleas affected only by mild to moderate changes, but middle turns of 6 cochleas affected by mild changes. Generally the nerve cells were seen in most cases having normal structure, occasionally some degenerated nerve cells were seen (Fig. 25, 26, 27) the cytoplasm was vacuolated, the nucleus became shrunken and piknotic. The cochlear nerve showed mild axonal degeneration of wallerian type; the degenerative changes were noted in the myelinated fibres in the osseous spiralis.

D- SPIRAL LIGAMENT AFFECTION

The spiral ligament and limbus spirals appeared normal without changes as in Fig. (22), (23).

Subdivision (B): (n = 9 animals = 18 cohclear specimens)

Examination of the cochlea of acute treated animals prepared by decalcification methods:

The specimens were stained also by previous staining as mentioned before and light microscopic photoswere presented for the organ of corti as in Fig. (19) for demonstration hair cells and supporting cells.

Also Fig. (24) shows stria vascularis which stained by H & E staining. Spiral ganglion cells demonstrated in Fig. (25, 26, 27), stained by silver and H & E staining. We observed from these photosthat there were great difference in clearance, fine details of the soft tissue archictecture and degenerative changes that occurred in hair cells, stria vascularis and spiral ganglion cells if we compared these photoswith those. Specimens which prepared by MMA embedding method which preserved the bone integrity and fine cellular structure so we got excellent interpretation about the proper degenerative changes in the specimens prepared by MMA embedding method. These findings were proved statistically through the tables (1,3,5,7,9) where we found out the significant difference was directed towards the MMA embedding method (new method).

Table (1): Number (No) and percent (%) distribution of cochleas affected by OHCs changes that observed after application of new and old methods of temporal bone embedding in group I (acute treated group).

Method	*Changes	1st turn		2nd turn		3rd turn	
		No.	%	No.	%	No.	%
New method	-ve	-	-	1	5.6	14	77.8
	Total +ve	18	100	17	94.4	4	22.2
Total		18	100%	18	100%	18	100%
Old method	-ve	2	12	5	27.8	18	100
	Total +ve	16	88	13	72.2	-	-
Total		18	100%	18	100%	18	100%
Z test		2.13		1.78		-	
P-value		< 0.05		< 0.05		-	
Significance		Significant		Significant		-	

* = Interpretation of fine changes that occurred in specimens.

(-ve → means that we cannot exactly interpretate changes).

(+ve → means that we can exactly interpretate changes).

Table (2): Number (No) and Percent (%) Distribution of affected cochleas by Degenerative Change of OHCs in 1st, 2nd and 3rd turns of cochleas in group I (Acute treated group).

Changes	1st		2nd turn		3rd turn	
	No.	%	No.	%	No.	%
-ve	2	5.6	6	16.7	32	88.9
1 + ve	2	5.6	12	33.3	4	11.1
2 + ve	6	16.7	18	50		
3 + ve	26	72.2	-	-		
Total	36	100%	36	100%	36	100%

$x^2 = 122.13$ P value < 0.0001 (Highly significant)

Table (3): Number (No) and percent (%) distribution of cochleas affected by IHCs changes that observed after application of new and old methods of temporal bone mebedding in group I (acute treated group).

Method	*Changes	1st turn		2nd turn		3rd turn	
		No.	%	No.	%	No.	%
New method	-ve	1	5.6	9	50	18	100
	Total +ve	17	94.4	9	50	-	-
Total		18	100%	18	100%	18	100%
Old method	-ve	9	50	17	94.4	18	100
	Total +ve	9	50	1	5.6	-	-
Total		18	100%	18	100%	18	100%
Z test		2.97		2.97		-	
P-value		< 0.001		< 0.001		-	
Significance		Significant		Significant		-	

Table (4): Number (No) and Percent (%) Distribution of cohcleas affected by degenerative Changes of IHCs in 1st, 2nd and 3rd Tums of Cochleas in Group I (Acute Treated Group).

Severity	1st		2nd turn		3rd turn	
	No.	%	No.	%	No.	%
-ve	10	27.8	26	72.2	36	100
1 + ve	16	44.4	8	22.2	-	-
2 + ve	8	22.2	2	5.6	-	-
3 + ve	2	5.6	-	-	-	-
Total	36	100%	36	100%	36	100%

$x^2 = 44.73$ $P < 0.0001$ (Highly significant)

Table (9): Number (No) and percent (%) distribution of cochleas affected by spiral ganglion cells after application of new and old methods of temporal bone embedding in group I (acute treated group).

Method	*Changes	1st turn		2nd turn		3rd turn	
		No.	%	No.	%	No.	%
New method	-ve	9	50	13	72.2	18	100
	Total +ve	9	50	5	27.8	-	-
Total		18	100%	18	100%	18	100%
Old method	-ve	17	94.4	17	94.4	18	100
	Total +ve	1	5.6	1	5.6	-	-
Total		18	100%	18	100%	18	100%
Z test		2.97		1.78		-	
P-value		< 0.001		< 0.05		-	
Significance		Significant		Significant		-	

Table (10): Number (No) and Percent (%) Distribution of cohcleas affected by Degenerative Changes of Spiral Ganglion Cells in the 1st, 2nd and 3rd Turns of the Cochleas in Group I (Acute Treated Group).

Changes	1st		2nd turn		3rd turn	
	No.	%	No.	%	No.	%
-ve	26	72.2%	30	83.3%	36	100%
1 + ve	8	22.2%	6	16.7%	-	-
2 + ve	2	5.6%	-	-	-	-
Total	36	100%	36	100%	36	100%

$x^2 = 13.08$ p value < 0.0001 (Highly significant)

Fig. (18): Photomicrograph of the cochlear basal turn of acute treated group prepared by (MMA). Showing degenerative changes in the organ of corti which was extensive, outer hair cell loss (1), Degeneration in supporting cells (2) and IHC (3). (H & E X 400).

Fig. (19): Photomicrograph of the cochlear basal turn of acute treated group prepared by decalcification showing degenerative changes in the organ of corti. (H & E X 400).

Fig. (20): Photomicrograph of the cochlear middle turn of the acute treated group prepared by (MMA) showing less degenerative changes in organ of corti as compared to basal turn. (H & E X 400)

Fig. (21): Photomicrograph of the cochlear basal turn of acute treated group prepared by (MMA). Showing complete destruction of organ of corti and supporting cells which were replaced by a layer of non differentiated curboid epithelium resting on the basilar membrane. (H & E X 400)

Fig. (22): Photomicrograph of the cochlear basal turn of acute treated group prepared by (MMA) showing degenerative changes that happened in the stria vascularis (S.V) cells but spiral ligament (SL) appeared normal.
(H & E X 400)

Fig. (23): Photomicrograph of the cochlear basal turn of acute treated group. Showing swallen and bulged marginal cells of stria vascularis into the endolymphatic space, and some cells ruptured (Masson stain X 400).

Fig. (24): Photomicrograph of the cochlea of acute treated group prepared by decalcification. Showing degenerative changes in stria vascularis (S.V) but spiral ligament appeared normal (SL). (H & E X 400).

Fig. (25): Photomicrograph of the cochlear basal turn of acute treated group prepared by MMA, showing spiral ganglion cells degeneration and loss.
(H & E X 400).

Fig. (26): Photomicrograph of the cochlear basal turn of acute treated group prepared by MMA, showing spiral ganglion cell degeneration (A) loss and mild axonal degeneration of nerve fibers (B) (Silver X 400).

Fig. (27): Photomicrograph of the cochlear of middle turn of acute treated group, showing normal cell pattern for spiral ganglion cells nerves (Silver X 400).

CHRONIC TREATED GROUP (GROUP II)

Subdivision (A): (n = 9 animals = 18 cochlear specimens)

Examination of the cochlea which prepared by methyl methacrylate embedding method:

A- ORGAN OF CORTI AFFECTION

1- Outer hair cell affection:

The degree of damage and number of missing of outer hair cells were lower than those in the acute treatment group. The main damage was observed at the base of the cochlea which correspond to the high frequency area. This pattern of distribution was discussed statistically through tables (11,12) which showed a significant difference for interpretation of fine changes of OHCs at basal, middle turns of examined cochlease in group II ($P < 0.001$, $P < 0.05$) respectively. Also taole 12 showed significant difference between 1st, 2nd and 3rd turns regarding OHCs changes ($P < 0.001$), also this table shows that 1st turn was highly affected followed by the 2nd one (61.1% and 22.2%) respectively from examined cochleas in group II. The degenerative changes in the remaining OHCs were less prominent than those specimens of acute treated group; in the form of vacuolization, rupture of plasmic membrane and picknosis of the nuclei, as in Fig. (28).

2- Inner hair cells affection:

The cells appeared almost normal except minimal vacuolization scattered in some IHCs limited only to the basal turn but there was no cell loss as in Fig. (28) and in tables (13,14), which revealed that new method had the upper hand in demonstration the fine changes in IHCs as it revealed 83.3% of cochleas affected but old method demonstrated only

16.7% of this changes and this result was significant ($P < 0.001$). Also table (14) showed statistical non significant difference between 1st, 2nd and 3rd turns regarding IHCs changes ($P > 0.05$) as there is no affection in 2nd, 3rd turns but the basal turns of (6 animals) were affected by mild to moderate degenerative changes.

3- Supporting cells affection:

The supporting cells that related to OHCs at the basal turn were affected by mild and moderate degree of swelling as in Fig. (28) and tables (15,16), which showed that new method had upper hand in detection of 12 cochlease affected by degenerative changes in supporting cells but the old method detected only 4 cochleas affected with fine changes at basal turns and this was significant value ($P < 0.001$).

Also table (16) revealed that the basal turns of 16 cochleas out of 36 were affected by mild affection (12 cochleas) and moderate (4 cochleas), but the middle turns of only 1 animal (2 cochleas) were affected by mild degenerative change and this of significant value ($P< 0.0001$).

B- STRIA VASCULARIS AFFECTION

Mild degree of stria vascularis affection only limited to the basal and middle turns in the form of mild marginal cells vacuolization but their nuclei appeared normal without lobulation, convolution or fragmentation as in acute treated specimens, as in Fig. (30) and in tables (17,18) which showed that new and old method were presented with non significant difference as the new method interpretated 4 cochlear affections by stria vascularis more than old one at the basal turns, but the

middle turns are equally presented by (2 cochlear affections for each one). Also table (18) presented with basal turns affection more than middle turns (33.3%, 11.2%) respectively and there was no affection of the stria vascularis of the apical turn (highly significant results as P-value < 0.0001).

C- SPIRAL GANGLION CELLS AFFECTION

The cells of spiral ganglion appeared normal in all cochlear specimens except 2 cochleas which were affected by mild pathologic changes at the basal turns (Fig. 32).

This result of no significant difference statistically if it was compared with the subgroup (B) in which the cochleas were prepared by decalcification ($P > 0.05$), as presented in table (19). Also table (20) showed no significant difference of distribution of degenerated spiral ganglion.

D- SPIRAL LIGAMENT AFFECTION

The spiral ligament and limbus spirals appeared normal without changes as in Fig. (28).

Subdivision (B): (n = 9 animals = 18 cochlear specimens)

Examination of the cochlea prepared by decalcification method:

The specimens were stained also by the previous staining as mentioned before and light microscopic fotos were presented for fine structures of the cochlea (organ of corti cells, stria vascularis, spiral ganglion cells) which demonstrated in Fig. (29), (31), (33) respectively.

We observed also as mentioned before that this traditional decalcification method was of lower value in interpretating of fine details of delicate soft tissue structure as those of organ of corti, stria vascularis and spiral ganglion. But on other hand the MMA embedding method had an upper hand in this demonstration and study of minimal changes that occurred in those delicate structures. These findings also were proved statistically through the tables (11, 13, 15, 17, 19) where we found out the significant difference was directed towards the MMA embedding method (new methods).

Table (11): Number (No) and percent (%) distribution of cochleas affected by OHCs changes that observed after application of new and old methods of temporal bone embedding in group II (chronic treated group).

Method	*Changes	1st turn		2nd turn		3rd turn	
		No.	%	No.	%	No.	%
New method	-ve	2	11/1	11	61.1	18	100
	Total +ve	16	88.9	7	38.9	-	-
Total		18	100%	18	100%	18	100%
Old method	-ve	12	66.6	17	94.4	18	100
	Total +ve	6	33.4	1	5.6	-	-
Total		18	100%	18	100%	18	100%
Z test		3.19		2.93		-	
P-value		< 0.001		< 0.05		-	
Significance		Significant		Significant		-	

Table (12): Number (No) and Percent (%) Distribution of cochleas affected by Degenerative Changes of OHCs in the 1st, 2nd and 3rd Turn of the Cochleas in Group II (Chronic Treated Group).

Changes	1st		2nd turn		3rd turn	
	No.	%	No.	%	No.	%
-ve	14	38.9%	28	77.8%	36	100%
1 + ve	12	33.3%	4	11.1%	-	-
2 + ve	8	22.2%	4	11.1%	-	-
3 + ve	2	5.6%	-	-	-	-
Total	36	100%	36	100%	36	100%

$x^2 = 35.53$ p value < 0.001 (significant).

Table (13): Number (No) and percent (%) distribution of cochleas affected by IHCs changes that observed after application of new and old methods of tempoal bone embedding in group II (chronic treated group).

Method	*Changes	1st turn		2nd turn		3rd turn	
		No.	%	No.	%	No.	%
New method	-ve	8	44.4	18	100	18	100
	Total +ve	10	55.6	-	-	-	-
Total		18	100%	18	100%	18	100%
Old method	-ve	16	88.9	18	100	18	100
	Total +ve	2	11.1	-	-	-	-
Total		18	100%	18	100%	18	100%
Z test		2.82		-		-	
P-value		< 0.001		-		-	
Significance		Significant		-		-	

Table (14): Number (No) and Percent (%) Distribution of cohcleas affected by Degenerative Changes of IHCs in the 1st, 2nd and 3rd Turns of the Cochleas in Group II (Chronic Treated Group).

Changes	1st		2nd turn		3rd turn	
	No.	%	No.	%	No.	%
-ve	24	66.7%	36	100%	36	100%
1 + ve	8	22.2%	-	-	-	-
2 + ve	4	11.1%	-	-	-	-
Total	36	100%	36	100%	36	100%

$x^2 = 27.0$ p value > 0.05 (Non significant)

Table (15): Number (No) and percent (%) distribution of cochleas affected by supporting cells changes that observed after application of new and old methods of temporal bone embedding in group II (chronic treated group).

Method	*Changes	1st turn		2nd turn		3rd turn	
		No.	%	No.	%	No.	%
New method	-ve	6	33.3	16	88.9	18	100
	Total +ve	12	66.7	2	11.1	-	-
Total		18	100%	18	100%	18	100%
Old method	-ve	14	77.8	18	100	18	100
	Total +ve	4	22.2	-	-	-	-
Total		18	100%	18	100%	18	100%
Z test		2.68		1.21		-	
P-value		< 0.001		> 0.05		-	
Significance		Significant		Non significant		-	

Table (16): Number (No) and Percent (%) Distribution of cochleas affected by Degeneration Changes of Supporting Cells in the 1st, 2nd and 3rd Turns of the Cochleas in Group II (Chronic Treated Group).

Changes	1st		2nd turn		3rd turn	
	No.	%	No.	%	No.	%
-ve	20	55.6%	34	94.4%	36	100%
1 + ve	12	33.3%	2	5.6%	-	-
2 + ve	4	11.1%	-	-	-	-
Total	36	100%	36	100%	36	100%

$X^2 = 30.78$ p value < 0.0001 (Highly significant)

Table (17): Number (No) and percent (%) distribution of cochleas affected by stria vascularis changes that observed after application of new and old methods of temporal bone embedding in group II (chronic treated group).

Method	*Changes	1st turn		2nd turn		3rd turn	
		No.	%	No.	%	No.	%
New method	-ve	10	55.6	16	88.9	18	100
	Total +ve	8	44.4	2	11.1	-	-
Total		18	100%	18	100%	18	100%
Old method	-ve	14	77.8	16	88.9	18	100
	Total +ve	4	22.2	2	11.1	-	-
Total		18	100%	18	100%	18	100%
Z test		1.41		1.36		-	
P-value		> 0.05		> 0.05		-	
Significance		Non significant		Non significant		-	

Table (18): Number (No) and Percent (%) Distribution of cochleas affected by Degenerative Changes of Stria Vascularis in the 1st, 2nd and 3rd Turns of the Cochleas in Group II (Chronic Treated Group).

Changes	1st turn		2nd turn		3rd turn	
	No.	%	No.	%	No.	%
-ve	24	66.7%	32	88.8%	36	100%
1 + ve	10	27.7%	2	5.6%	-	-
2 + ve	2	5.6%	2	5.6%	-	-
Total	36	100%	36	100%	36	100%

$x^2 = 18.43$ p value < 0.0001 (Highly significant).

Table (19): Number (No) and percent (%) distribution of cochleas affected by spiral ganglion cells changes that observed after application of new and old methods of temporal bone embedding in group II (chronic treated group).

Method	*Changes	1st turn		2nd turn		3rd turn	
		No.	%	No.	%	No.	%
New method	-ve	16	88.9	18	100	18	100
	Total +ve	2	11.1	-	-	-	-
Total		18	100%	18	100%	18	100%
Old method	-ve	18	100	18	100	18	100
	Total +ve	-	-	-	-	-	-
Total		18	100%	18	100%	18	100%
Z test		1.21		-		-	
P-value		> 0.05		-		-	
Significance		Non significant		-		-	

Table (20): Number (No) and Percent (%) Distribution of chochleas affected by Degenerative Changes of Spiral Gangtion Cells in the 1st, 2nd and 3rd Turns of the Cochleas in Group II (Chronic Treated Group).

Changes	1st turn		2nd turn		3rd turn	
	No.	%	No.	%	No.	%
-ve	34	94.4%	36	100%	36	100%
1 + ve	2	5.6%	-	-	-	-
Total	36	100%	36	100%	36	100%

$x^2 = 4.05$ P-value > 0.05 (Non significant).

Fig. (28): Photomicrograph of the cochlear basal turn of chronic treated group prepared by MMA, showing mild degenerative changes in OHCs (1) and supporting cells related to it (2) But inner hair cells normal (3) and normal spiral ligament (4).

(H & E X 400).

Fig. (29): Photomicrograph of the cochlear basal turn of chronic treated group, prepared by decalcification method. Showing degenerative changes that occurred in OHCs (1) and supporting cells related to it (2) and mild degeneration in IHC (3), normal spiral ligament (4).

(H & E X 400).

Fig. (30): Photomicrograph of the cochlear basal turn of chronic treated group prepared by (MMA), showing mild stria vascularis affection, limited to marginal cells (Arrow) and spiral ligament is normal.
(H & E X 400)

Fig. (31): Photomicrograph of the cochlear basal turn of chronic treated group prepared by decalcification, showing stria vascularis (S.V.) and spiral ligament (SL) (H & E X 400).

Fig. (32): Photomicrograph of the cochlear basal turn of chronic treated group prepared by (MMA), showing normal pattern of spiral ganglion cells and nerve fibers (Silver Stain X 400).

Fig. (33): Photomicrograph of the cochlear basal turn of chronic treated group prepared by decalcification showing normal pattern of spiral ganglion cells and nerve fibers (Silver X 400).

STATISTICAL ANALYSIS OF RESULTS

COMPARISON BETWEEN STUDY GROUPs

Table (21-a): Number (No) and Percent (%) Distribution of Degerative Changes of OHCs at basal Turns in Both Acute Treated Group (Group I) and Chronic Treated Group (Group II).

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	2	5.6%	14	38.9%	16	22.3%
1 + ve	2	5.6%	12	33.3 %	14	19.4%
2 + ve	6	16.7%	8	22.2%	14	19.4%
3 + ve	26	72.2%	2	5.6%	28	38.9%
Total	36	100%	36	100%	72	100%

$X^2 = 38.83$ P-value < 0.0001 (Highly significant)

This table showed a highly statistical significant difference between group I and II regarding OHCs degenerative changes at basal turns ($P < 0.001$); this significance was directed toward group I i.e., acute treated group 72.2% of basal turns in cochleas of group I affected by severe changes of OHCs. Also there was only 5.6% of group II affected by severe changes.

Table (21-b):

Change	Group I		Group II		Total		X^2	P value	OR*	95% CI**
	No.	%	No.	%	No.	%				
No changes	2	5.6%	14	38.9%	16	22.2%	9.72	< 0.001	10.82	2.02-76-7
Total changes	34	94.4%	22	61.1%	56	77.8%				
Total	36	100%	36	100%	72			Significant		

* Odds ratio.

** 95% confidence interval.

This table showed that group I had 10 folds increase in the occurance of OHCs degenerative changes – at basal turn – to that of group II (95% CI = 2.02 – 76.7).

Table (22-a): Number (No) and percent (%) Distribution of Degenerative Changes of IHCs at Basal Turns in Both Group I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	10	27.8%	24	66.7%	34	47.2%
1 + ve	16	44.4%	8	22.2%	24	33.3%
2 + ve	8	22.2%	4	11.1%	12	16.7%
3 + ve	2	5.6%	-	-	2	2.8%
Total	36	100%	36	100%	72	100%

$X^2 = 11.76$ P-value < 0.001 (Significant)

This table showed statistical significant difference between group I, II regarding IHCs affection at Basal turn ($P < 0.001$). This significance was directed towards group I, as it was affected in 72.2% of its cochlear specimens but 33.3% in group II was affected by mild and moderate changes.

Table (22-b):

Change	Group I		Group II		Total		X^2	P value	OR*	95% CI**
	No.	%	No.	%	No.	%				
- ve	10	27.8	24	66.7	34	47.2	9.42	< 0.0001	5.20	1.71-16.29
+ ve	26	72.2	12	33.3	38	52.8				
Total	36	100%	36	100%	72	100%		H.S*		

* = Highly significant

This table showed that group I had 5 folds increase in the occurrence of degenerative changes for IHCs-at basal turns-to that of group II (95% CI = 1.71 – 16.29)

Table (23-a): Number (No) and percent (%) distribution of degenerative changes of supporting cells at basal turns in both group I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	4	11.1	20	55.6	24	33.3
1 + ve	12	33.4	12	33.3	24	33.3
2 + ve	18	50	4	11.1	22	30.6
3 + ve	2	5.5	-	-	2	2.8
Total	36	100%	36	100%	72	100%

$X^2 = 21.57$ P-value < 0.0001 (Highly significant)

This table revealed a highly significant difference between group I and group II regarding supporting cells at basal turns ($P < 0.0001$) this significance was directed towards group I.

Table (23-b):

Change	Group I		Group II		Total		X^2	P value	OR	95% CI
	No.	%	No.	%	No.	%				
- ve	4	11.1	20	55.6	24	33.3	14	< 0.001	10.0	2.6-41.9
+ ve	32	88.9	16	44.4	48	66.7				
Total	36	100%	36	100%	72	100%		H.S*		

*H.S = Highly significant

This table showed that group I had 10 folds increase in the occurrence of degenerative changes for supporting cells- at basal turns- to that of group II (95% CI = 2.6 – 41.9).

Table (24-a): Number (No) and percent (%) distribution of degeneration changes of stria vascularis at basal turns in both group I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	2	5.6	24	66.7	26	36.1
1 + ve	8	22.2	10	27.7	18	25
2 + ve	16	44.4	2	5.6	18	25
3 + ve	10	27.8	-	-	10	13.9
Total	36	100%	36	100%	72	100%

$X^2 = 39.72$ P-value < 0.001 (Highly significant)

This table showed a highly statistical difference between group I, group II regarding stria vascularis at basal turns ($P < 0.0001$). This significance was directed towards group I as (94.4% and 33.3%) of cochlear specimens were affected in group I and group II respectively.

Table (24-b):

Change	Group I		Group II		Total		X^2	P value	OR	95% CI
	No.	%	No.	%	No.	%				
- ve	2	5.6	24	66.7	26	36.1	56.55	< 0.0001	34.00	6.2 – 245.7
+ ve	34	94.4	12	33.3	46	63.9				
Total	36	100%	36	100%	72	100%		H.S		

This table showed that group I had 34 folds increase in the degenerative changes for stria vascularis at basal turns to that of group II (95% CI = 6.2 – 245.7).

Table (25-a): Number (No) and percent (%) distribution of degenerative changes of spiral ganglion cells at basal turns of both group I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	26	72.2	34	94.4	60	83.3
1 + ve	8	22.2	2	5.6	10	13.9
2 + ve	2	5.6	-	-	2	2.8
Total	36	100%	36	100%	72	100%

$X^2 = 6.66$ P-value < 0.05 (Significant)

This table showed a significant difference between group I and group II regarding spiral ganglion cells at basal turns ($P < 0.05$). This significance was directed towards group I as it was affected by 27.8% but group II affected only by 5.6% in its specimens.

Table (25-b):

Change	Group I		Group II		Total		X^2	P value	OR*	95% CI**
	No.	%	No.	%	No.	%				
- ve	26	72.2	34	94.4	60	83.3	4.9	< 0.05	6.54	1.18-47.5
+ ve	10	27.8	2	5.6	12	16.7				
Total	36	100%	36	100%	72	100%		Significant		

This table showed that group I had 6 folds increase in occurrence of degenerative changes for spiral ganglion cells at basal turns – to that of group II (95% CI = 1.18 – 47.5).

Table (26-a): Number (No) and percent (%) distribution of degeneration changes of OHCs at middle turns of both group I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	6	16.7	28	77.8	34	47.2
1 + ve	12	33.3	4	11.1	16	22.2
2 + ve	18	50	4	11.1	22	30.6
Total	36	100%	36	100%	72	100%

$X^2 = 0.01$ P-value > 0.05 (Non significant)

This table showed a non significant difference between group I and group II regarding OHCs affection at middle turns (P > 0.05). But the affection presented in group I more than group II.

Table (26-b):

Change	Group I		Group II		Total		X^2	P value	OR	95% CI
	No.	%	No.	%	No.	%				
- ve	6	16.7	28	77.8	34	47.2	24.6	< 0.001	17.5	4.74- 69.4
+ ve	30	83.3	8	22.2	38	52.8				
Total	36	100%	36	100%	72	100%		Significant		

This table showed that group I had 17 folds increase in occurrence of degenerative changes for OHCs- at middle turns to that of group II (95% CI = 4.74 – 69.4).

Table (27-a): Number (No) and percent (%) distribution of degenerative changes of IHCs at middle turns of both group I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	26	72.2	36	100	62	86.1
1 + ve	8	22.2	-	-	8	11.1
2 + ve	2	5.6	-	-	2	2.8
Total	36	100%	36	100%	72	100%

This table showed 27.8% of cochlear specimens were affected in group I but there was no affection in group II regarding IHCs at middle turns.

Table (27-b):

Change	Group I		Group II		Total		X^2	P value	OR	95% CI
	No.	%	No.	%	No.	%				
- ve	26	72.2	36	100	62	86.1	9.4	< 0.001	-	-
+ ve	10	27.8	-	-	10	13.9				
Total	36	100%	36	100%	72	100%		Significant		

This table showed statistical significant difference between group I and group II regarding the total changes affected IHCs at middle turns ($P < 0.001$).

Table (28-a): Number (No) and percent (%) distribution of degenerative changes of supporting cells at middle turns in both group I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	4	11.1	34	94.4	38	52.8
1 + ve	24	66.7	2	5.6	26	36.1
2 + ve	6	16.6	-	-	6	8.3
3 + ve	2	5.6	-	-	2	2.8
Total	36	100%	36	100%	72	100%

X2 = 6.04 P-value < 0.05 (significant)

This table showed a significant difference between group I and group II regarding supporting cells at middle turns ($P < 0.05$). This significance was directed towards group I because 88.9% from its cochlear specimens were affected but 5.6% of cochlear specimens were only affected in group II.

Table (28-b):

Change	Group I		Group II		Total		X^2	P value	OR	95% CI
	No.	%	No.	%	No.	%				
- ve	4	11.11	34	94.4	38	52.8	46	< 0.0001	136	19.48-1308.4
+ ve	32	88.9	2	5.6	34	47.2				
Total	36	100%	36	100%	72	100%		Highly significant		

This table showed that group I had 136 folds increase in the occurrence of degenerative changes for supporting cells- at middle turns to that of group II (95% CI = 19.48 – 1308.4).

Table (29-a): Number (No) and percent (%) distribution of degenerative changes of stria vascularis at middle turns in both group I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	12	33.3	32	88.8	44	61.1
1 + ve	14	38.9	2	5.6	16	22.2
2 + ve	10	27.8	2	5.6	12	16.7
Total	36	100%	36	100%	72	100%

$X^2 = 11.76$ P-value > 0.05 (Non significant)

This table showed a non significant difference between group I and group II regarding stria vascularis at middle turns (P >0.05). But the affection was presented in specimens of group I more than group II (66.7% : 11.2%) respectively.

Table (29-b):

Change	Group I		Group II		Total		X^2	P value	OR	95% CI
	No.	%	No.	%	No.	%				
- ve	12	33.3	32	88.8	44	61.1	21.1	< 0.0001	16	4.06-69.02
+ ve	24	66.7	4	11.2	28	38.9				
Total	36	100%	36	100%	72	100%		Highly significant		

This table showed that group I had 16 folds increase in the occurrence of degenerative changes for stria vascularis – at middle (turns to that of group II (95% CI = 4.06 – 69.2).

Table (30): Number (No) and percent (%) distribution of degenerative changes in spiral ganglion cells at middle turns of group I and group II.

Change	Group I		Group II		Total		X^2	P value
	No.	%	No.	%	No.	%		
- ve	30	83.3	36	100	66	91.7	4.55	< 0.05
+ ve	6	16.7	-	-	6	8.3		
Total	36	100%	36	100%	72	100%		Significant

This table showed a significant difference between group I and group II regarding spiral ganglion cells affection at middle turns ($P<0.05$) this significance was directed towards group I.

Table (31): Number (No) and percent (%) distribution of degenerative changes of OHCs at 3rd turn of group I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	32	88.8	36	100	68	94.4
1 + ve	4	11.2	-	-	4	5.6
Total	36	100%	36	100%	72	100%

$X^2 = 2.38$ P-value > 0.05 (non significant)

This table showed non significant difference between group I and group II regarding OHCs affection at 3rd turns (P > 0.05).

Table (32-a): Number (No) and percent (%) distribution of degenerative changes of supporting cells at 3rd turns of groups I and group II.

Changes	Group I		Group II		Total	
	No.	%	No.	%	No.	%
- ve	32	88.8	36	100	68	94.4
1 + ve	2	5.6	-	-	2	2.8
2 + ve	2	5.6	-	-	2	2.8
Total	36	100%	36	100%	72	100%

Table (32-b):

Change	Group I		Group II		Total		X^2	P value
	No.	%	No.	%	No.	%		
- ve	32	88.8	36	100	68	94.4	2.38	> 0.05
+ ve	4	11.2	-	-	4	5.6	-	
Total	36	100%	36	100%	72		Non significant	

This tables showed a non significant difference between group I and group II regarding supporting cells at 3rd turns (P > 0.05).

Table (33): Number (No) and percent (%) distribution of degenerative changes of stria vascularis at 3rd turns of group I and group II.

Change	Group I		Group II		Total		X^2	P value
	No.	%	No.	%	No.	%		
- ve	32	88.9	36	100	68	94.4	2.38	> 0.05
+ ve	4	11.1	-	-	4	5.6		
Total	36	100%	36	100%	72	100%	Non significant	

This table showed no significant difference between group I and group II regarding stria vascularis affection at 3rd turns ($P > 0.05$).

N.B.:

There were no changes at apical turns in group I and group II regarding IHCs and spiral ganglion cells, so I did not mention tables for it.