BIOCHEMICAL ASSESSMENT OF AMNIOTIC MEMBRANE EXTRACT

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Introduction

Amniotic membrane (AM) is a biologically viable structure with many properties that made it a popular choice in regenerative medicine. It has low immunogenicity, reepithelialization effects, anti-inflammatory, nontumorigenic and antimicrobial properties. These pleiotropic capabilities are due, in part, to its ability to synthesize and release biologically active chemicals such as growth factors. Amniotic membrane extract (AME) is one of the many modifications that are done on the amniotic membrane offering a much simpler and convenient approach. Despite being perceived as a single entity; amniotic membrane has many subregional differences proved histologically and so it stands to reason that the content of the biological factors differs as well according to the region. Knowledge on specific factors could facilitate the decision on which region to select for a specific application. Transforming growth factor-beta and Hepatocyte growth factor are of particular interest given their intimate involvement in all phases of wound healing.

Aim of the Work

The aim of this study was to assess the total protein content and concentration of Hepatocyte Growth Factor (HGF) and Transforming Growth Factor-Beta1 (TGF-β1) in amniotic membrane extract for their therapeutic uses and compare their concentrations between placental and reflected regions of AM.

Subjects and Methods

SUBJECTS: This study was conducted on 40 placentas of healthy women delivered by elective cesarean section at term. Subjects were selected from the Obstetrics Ward at the Shatby University Hospital of Alexandria.

METHODS: Blunt dissection of the amnion from the chorion was done with labelling of the placental and reflected amnion.

The amnions were washed then incubated in sterile saline solution for hours.

It was then cut into pieces, submerged into liquid nitrogen pulverized into powder and then homogenized in Buffered phosphate solution (BPS) with protease inhibitor and finally the solution was sonicated on ice, centrifuged, the supernatant was collected and centrifuged again, finally producing the AME.

Total protein content for both reflected and amniotic membrane was measured using Nanodrop™ 2000c spectrophotometer and compared.

TGF-B concentration for both regions was measured using DRG® TGF-ß1 ELISA kit and compared.

HGF concentration for both regions was measured using Quantikine® Human HGF ELISA kit and compared.

Results

The concentration of TGF-β1 in placental AME was 1092 ± 450 pg/ml while that of reflected AME was 1225 ± 715 pg/ml. There was a statistical significance between TGF-β1 content in reflected AME and placental AME (p = 0.047) with the concentration being higher in reflected. The concentration of HGF in placental AME was 42,075 ± 33453.29 pg/ml, while the concentration in reflected AME was 30,988.75 ± 34729.41 pg/ml. There was a statistical difference between the HGF content in the placental AME and the reflected AME (P=0.025) with the concentration being higher in placental.

Table 1: Comparison between placental and reflected HGF (n = 40)

	Placental HGF	Reflected HGF	Z	p
Min. – Max.	2000 – 100000	300 – 110000		
Mean ± SD	42075 ± 33453.29	30988.75 ± 34729.41	-2.240	0.025*

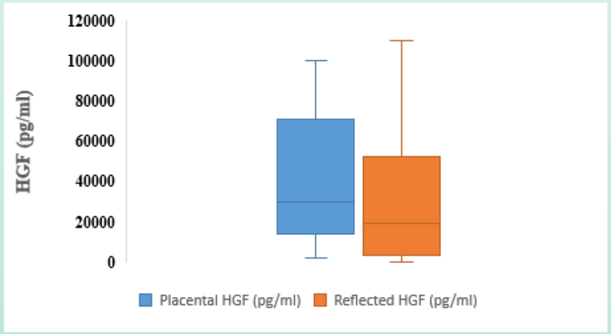

Figure 1 Comparison between placental and reflected HGF (n = 40)

Table 2: Comparison between placental and reflected TGF-β1 (n = 40)

TGF-B (pg/ml)	Placental	Reflected	Z	p
Min. – Max.	575 – 2994	513 – 4067	1.983*	0.047*
Mean ± SD.	1092 ± 450	1225 ± 715		

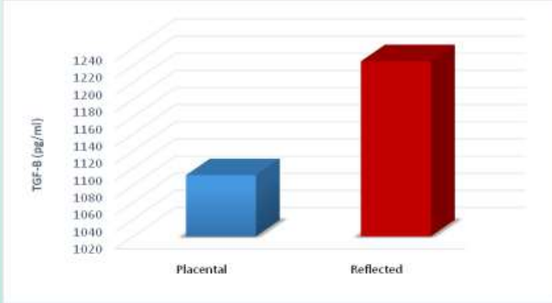

Figure 2 Comparison between placental and reflected TGF-β1 (n = 40)

Conclusion

- Different preparation and handling techniques can affect the protein content of the extract which may cause some biological factors to be inadvertently lost or retained.
- Biological factors content differs according to the region of the membrane.
- Reflected amnion showed a higher concentration of TGF-B1 making it a more appropriate choice for application in diabetic foot ulcer and venous ulcers.
- Placental amnion showed higher concentration of HGF and relatively poor concentration of TGF-B1 making it a more appropriate choice in ophthalmological application.