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Assessment of selected biochemical and immunological parameters after treatment by Cosmetic Procedures

Abdullah Salim Al-Karawi [1,2,3], Ali Mahmoud Atoom[1,3], Safwan Mahmoud Al-Adwan[1,3], Sofia Wasfi Adwan[4*]

[1]Al-Ahliyya Amman University, Al-Salt, Jordan
[2]Medical Laboratory Technologies, Al-Hadi University College, Baghdad, Iraq
[3]Pharmacological and Diagnostic Research Center, Al-Ahliyya Amman University, Amman, Jordan
[4] American University of Madaba, Madaba, Jordan

***Corresponding author:** Sofia Wasfi Adwan, American University of Madaba, Madaba, Jordan, Email: s.adwan@aum.edu.jo

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ABSTRACT

Background: The use of non-invasive cosmetic procedures such as Botulinum toxin and hyaluronic acid filler (HA) are commonly used in cosmetic and therapeutic. However, BOTOX therapy is generally considered safe and without significant adverse effects. However, these treatments' impacts must be understood and investigated thoroughly. The objective of this prospective study was to evaluate the impact of using injections of hyaluronic acid filler (HA) and BOTOX on some human biochemical parameters, CBC, and inflammatory markers.

Methods: Eighty-five subjects who have undergone BOTOX and/or HA injections were recruited. Samples of study and control groups were collected into EDTA for CBC and serum tubes for the analysis of the rest of the parameters.

Results: Our results revealed that using BOTOX and HA filler has no significant effect on ALT, AST, GGT, and CK-MB levels (p-value > 0.05). While there was a decrease in ALP level in subjects injected with both BOTOX and HA filler. Moreover, we discovered that two of our study groups had significantly higher serum LDH levels than the control group. CRP, TNF-α, and IL-17 were elevated in the study group compared with the control group. However, this difference was statistically insignificant (p-value > 0.05).

Conclusion: Accordingly, using BOTOX and HA filler may result in mild changes in clinical laboratory tests. Future investigations are now necessary to highlight the influence of these cosmetic procedures on various clinical laboratory parameters.

Keywords: *effect, botulinum toxin, BOTOX, hyaluronic acid, biochemical parameters, hematological parameters, immunological parameters*

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INTRODUCTION

Medical cosmetic technology is rapidly advancing, causing a significant development of new procedures to improve aesthetic and functional problems with greater simplicity. Non-surgical cosmetic procedures are becoming a widespread alternative practice in cosmetics and therapy. Patients' awareness of these growing alternatives has increased demand for this growing range of new treatments (1, 2). Botulinum toxin, fillers, chemical peels, non-surgical laser treatment, and other noninvasive procedures are used mainly to prolong youthfulness (3). The most commonly used aesthetic procedures nowadays for both genders are botulinum toxin (BOTOX) and hyaluronic acid (HA) (4).

BOTOX is produced from a toxin formed by Clostridium botulinum bacteria, a gram-positive, rod-shaped, spore-forming, strictly anaerobic bacteria (5). The Botulinum toxin consists of a mixture of proteins comprised of nontoxic proteins and the botulinum neurotoxin (BoNT) (6). Although the therapeutic use of BOTOX has been chiefly associated with cosmetic purposes, its benefits and services go beyond such decorative applications. The primary therapeutic use of Botulinum toxins depends on their ability to relax muscles by restricting acetylcholine release at the neuromuscular junction (7). However, treatment with BOTOX is widely recognized as safe, practical, and mainly lacking any critical side effects. Unfortunately, this is untrue because adverse effects from BOTOX injections, such as discomfort, edema, ecchymosis, erythema, and temporary hypoesthesia, can occur anywhere. Additionally, systemic botulinum toxin diffusion and more severe and widespread side effects from BOTOX injections are possible (8). Hence, it is essential to learn about all possible adverse consequences, their processes, and prevention methods (8).

Hyaluronic acid (HA) injectable fillers are usually used for a range of conditions, mainly to reduce the marks of facial aging and wrinkles by their ability to hold collagen and elastin in the correct configuration (9) also to replace soft tissue loss and reduce scars (10). The unique structure of HA gives it a remarkable ability to hold almost 1000 times its weight of water (11). The HA is a highly hydrophilic substance, forms gels at even low concentrations, and can withstand high compressive forces. It is mainly made from the synthetic fermentation of the Staphylococcus equine bacterium, regarded as purer than any other source, and shows less allergic reaction (12). HA is naturally occurring and typically found in skin, cartilage, bone, synovial fluid, and connective tissues (13). HA plays a significant role in regulating diverse biological processes and maintaining homeostasis in the body. However, with age, the amount of HA in the native tissue decreases, leading to decreased dermal hydration and increased folding (11). HA action is achieved by improving skin hydration, soft tissue augmentation, collagen stimulation, and face rejuvenation (9). Furthermore, its antioxidant effect leads to decreased wrinkle formation and recovered deep fine lines (14). The cosmetic use of HA positively affects the quality of life reflected in the self-esteem level and self-image after HA facial rejuvenation (15, 16).

HA filler injections used to enhance lip fullness indicated that HA is safe and effective (17). Consistent with these results, 93% of participants in the HA face microinjection procedure felt satisfied with no remarkable adverse effects (18). HA dermal fillers have evolved in recent years, and some complications have been associated with their use. The most common side effects of HA injections are local injection-related side effects such as discomfort, edema, redness, minor bruising, swelling, soreness, erythema, itching, and ecchymosis (19). Vascular occlusion was the most severe complication associated with HA filler injections. It can be a localized or a distant occlusion that may cause blindness, or cerebral ischemic events can occur. If not treated correctly, the affected area will develop reticulated erythema, purpura, ulceration, and, consequently, scarring (20). Another complication is infections which may be viral, bacterial, or fungal (21).

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The safety and efficacy of injectable HA fillers combined with BOTOX have also been tested for the treatment of wrinkles, signs of facial congestion, dry eyes, topical swelling, and headache were measured. Results showed that 96.5% of subjects showed an excellent tolerance to the treatment, a need for a lower dose, and high satisfaction levels (22).

Considering these findings, the primary goal of this study is to examine the impact of non-surgical cosmetic procedures, mainly BOTOX and HA filler, on some biochemical parameters related to liver function (LFT), cardiac enzymes, complete blood count (CBC), Creatinine, and some essential inflammatory parameters such as C-reactive protein (CRP), lactate dehydrogenase (LDH), Tumor Necrosis Factor-alpha (TNF-α), and Interleukin 17 (IL-17). To achieve this aim, we screened Jordanian patients undergoing any of these procedures to assess the difference in their blood biochemical, immunological, and hematological parameters if present. To our knowledge, this is the first thorough investigation into how BOTOX and HA fillers affect various blood parameters. This work will enhance our understanding of the involvement of such procedures in the pathogenesis of different body organs reflected through the blood as mentioned above parameters. Also, it will help shed light on the safety of these procedures and provide healthcare providers with appropriate information regarding these aesthetic procedures.

MATERIALS AND METHODS

This prospective study examines biochemical, hematological, and inflammatory human laboratory parameters to determine the impact of BOTOX and HA filler injections.

Study participants and ethical considerations

Subjects who have undergone BOTOX and/or HA injections 2-4 times in the last three years were recruited from a Dermatology clinic in Jordan. Patients' samples were collected before injecting into EDTA for CBC and serum tubes for the rest of the parameters. All participants acknowledged the aim of the study and the procedure. The risks and benefits of the study were also clearly explained, and who subsequently gave their informed consent in accordance with the Helsinki declaration prior to sample collection. Also, all the data collected from the study were handled with strict confidentiality. The ethical committee approved this study at Al-Ahliyya Amman University-Deanship of Scientific Research University (reference number: IRB; AA-2-3-21).

To participate in this study, participants had to meet the following requirements:

Adult Jordanian volunteers.

Male or female aged between 18-68.

Individuals in the study group should have gotten at least one dose of BOTOX and/or HA filler injection(s), whereas the control group should not have received any.

Every participant in our study who suffered from a chronic disease was prohibited. Subjects were categorized into two major groups: control and study groups. The study group was further sub-categorized according to the injection they received as the following: Group 1 "only BOTOX": patients who have been injected with BOTOX only, group 2 "only filler": patients who have been injected with HA filler only; group 3 "BOTOX-HA": patients who have been injected with both BOTOX and HA filler, in addition to a control group that includes healthy subjects that have never undergone any cosmetic procedure.

METHODOLOGY

According to the manufacturer's instructions, biochemical tests were performed using automated clinical chemistry instruments, including calibrators, controls, and reagents. All reagent kits used were purchased from Human diagnostics (Wiesbaden, Germany). The methodology used differs according to the test as follows: ALT (pro. No. 12012), AST (pro. No. 12011), and ALP (pro. No. 12017), which are based on the Modified IFCC method,

Colorimetric test Persijn/van der Slik method for the GGT test (pro. No. 12013), Colorimetric Cr test (pro. No. 10052), Humazym M-test for CK-MB (pro. No. 12008), LDH is based on modified SCE method (pro. No. 12214) and last but not least Immunoturbidimetric test for the CRP test (pro. No. 11241). The previous tests were performed for the sera of all participants with the HumaStar 200 Instrument (Human diagnostics, Wiesbaden, Germany). The automated hematology analyzer did CBC Measurement for whole blood electronically (Celltac Alpha MEK-6500K, Nihon Kohden, Japan). Moreover, IL-17 (pro. No. DY317, R&D systems, Bio-Techne, Minneapolis, U.S.) and TNF-α (pro. No. DY210, R&D systems, Bio-Techne, Minneapolis, U.S.) serum levels were measured with BioTek Instrument (Vermont, U.S.). A sandwich ELISA technique was used to determine IL-17 and TNF-α levels, where a duplicated run was performed for all samples and read at 450 nm. All analyses were performed as per the manufacturers.

Data analysis

Software called Graph-Pad Prism version 8.0 was used to analyze the data. Data were expressed as mean ± SD; descriptive statistics were used to summarize the historical and medical characteristics of the study participants. Furthermore, an unpaired student's t-test was conducted to determine the significant difference between the study and the control groups concerning biochemical, hematological, and inflammatory parameters. Data were considered significant at P-values $\leq$ 0.05. All of the parameters mentioned above were compared between all groups using one-way ANOVA.

RESULTS

Participants' characteristics

Initially 130 subjects were recruited in the study, however 21 patients were found to have a chronic disease and then were excluded. A total of 109 (12 males, 97 female) subjects were enrolled and subsequently categorized into two major groups. Eighty-five of participants have undergone BOTOX and/or filler injections and were designated to the study group, and 24 healthy subjects who haven't undergone any aesthetic procedure were designated to the control group. The study group's participants have subsequently separated into three groups: 18 patients received BOTOX injections alone in the first group, 8 patients received filler injections alone in the second group, and 59 patients received both BOTOX and HA injections in the third group. There was a female predominance of 97 out of the 109 participants (89%). The average age of all the 85 volunteers in the study group was 40.5 years. While the average age of individuals in the control group was 38.9 years. The p-value for age and gender was insignificant between the control and study groups indicating that both variables had no effect on the results of our study.

The effect of "only BOTOX" on laboratory parameters.

Biochemical

We found that there was no significant difference between the control and the study group (p-value > 0.05) in the ALP, ALT, AST, GGT, CRP, and CK-MB levels (Table 1). However, there was a significant decrease in serum creatinine level within the study group who received "only BOTOX" compared with the control group (Figure 1B). Also, we found there that there was a significant increase in serum LDH level within the study group who received "only BOTOX" compared with the control group (Figure 1F).

Immunological

Our results also showed that there was an increase in the TNF-α and IL-17 levels in our study group. Nonetheless, it was statistically insignificant (p-value > 0.05) (Table 1).

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TABLE 1. Effect of "only BOTOX" injections on biochemical and immunological parameters

biochemical and immunological parameters			
Parameter	**Mean of control group** * **(U/L)**	**Mean of study group** † **(U/L)**	**P-value**
ALP	70.38 ± 20.56	69.22 ± 43.12	> 0.05
ALT	13.58 ± 6.51	14.61 ± 8.63	
AST	18.58 ± 6.37	18.61 ± 6.66	
GGT	10.75 ± 10.38	16.00 ± 17.58	
Creatinine	0.87 ± 0.16	0.72 ± 0.19	0.01 ‡
CRP	2.65 ± 1.24	4.04 ± 4.52	> 0.05
LDH	316.90 ± 118.70	464.80 ± 165.90	0.01 ‡
CK-MB	13.42 ± 7.80	10.61 ± 3.96	> 0.05
TNF-α	9.19 ± 2.67	60.34 ± 142.60	
IL-17	6.39 ± 2.99	13.30 ± 22.46	
* Data represents the average of 24 participants ±SD † Data represents the average of 18 participants ±SD ‡ Statistically significant			

Hematological

There, we discovered that there was no discernible difference between the two groups in any of the hematological parameters (Table 2). However as compared to the control group, the hemoglobin level in the study group that received "only BOTOX" injections was significantly lower the P-value 0.01 (Figure 3A)

TABLE 2. Effect of "only BOTOX" injections on Hematological parameters

Hematological parameters			
Parameter	Mean of control group *	Mean of study group †	P-value
Hb (g/dl)	13.49 ± 1.39	12.21 ± 1.11	0.01 ‡
RBCs ($\times 10^{12}$/L)	4.99 ± 0.44	4.76 ± 0.25	> 0.05
RDW (%)	13.03 ± 0.79	13.34 ± 1.27	
WBCs ($\times 10^{9}$/L)	6.94 ± 1.50	6.41 ± 1.53	
Neutrophils (%)	56.79 ± 7.91	58.83 ± 8.50	
Lymphocytes (%)	42.00 ± 7.59	39.00 ± 8.46	
Monocytes (%)	1.25 ± 0.44	1.17 ± 0.38	
Eosinophils (%)	1.21 ± 0.41	1.00 ± 0.00	
Platelets ($\times 10^{9}$/L)	246.90 ± 63.55	229.80 ± 56.84	
* Data represents the average of 24 participants ±SD † Data represents the average of 18 participants ±SD ‡ Statistically significant			

The effect of "only Filler" injections on laboratory parameters

Biochemical

As demonstrated in (Table 3), when the biochemical parameters (ALP, ALT, AST, GGT, creatinine, CRP, LDH, and CK-MB) levels were analyzed for both "only filler" and control groups, all of the aforementioned parameters showed no significant difference between the two groups (p-value > 0.05), as was observed.

Immunological

Although levels of TNF-α and IL-17 levels were higher in the "only filler" study group compared with the control group. However, this difference was statistically insignificant (p-value > 0.05) (Table 3)

TABLE 3. Effect of "only Filler" injections on biochemical and immunological parameters

Biochemical and immunological parameters			
Parameter	**Mean of control group * (U/L)**	**Mean of study group † (U/L)**	**P-value**
ALP	70.38 ± 20.56	68.13 ± 19.48	> 0.05
ALT	13.58 ± 6.51	15.63 ± 9.29	
AST	18.58 ±6.37	18.25 ± 7.23	
GGT	10.75± 10.38	37.25 ± 80.79	
Creatinine	0.87 ± 0.16	0.81 ± 0.21	
CRP	2.65 ± 1.24	2.27 ± 1.84	
LDH	316.90 ± 118.70	400.10 ± 192.20	
CK-MB	13.42 ± 7.80	10.75 ± 5.57	
TNF-α	9.19 ± 2.67	59.80 ± 73.52	
IL-17	6.39 ± 2.99	9.09 ± 6.01	
* Data represents the average of 24 participants ±SD † Data represents the average of 18 participants ±SD			

Hematological

As shown in (Table 4), there was no significant difference between both groups in all the hematological parameters (p- value >0.05).

TABLE 4. Effect of "only Filler" injections on hematological parameters.

Hematological parameters			
Parameter	**Mean of control group ***	**Mean of study group †**	**P-value**
Hb (g/dl)	13.49 ± 1.39	12.73 ± 1.28	> 0.05
RBCs ($\times 10^{12}$/L)	4.99 ± 0.44	4.86 ± 0.46	
RDW (%)	13.03 ± 0.79	13.18 ± 1.07	
WBCs ($\times 10^{9}$/L)	6.94 ± 1.50	6.56 ± 1.60	
Neutrophils (%)	56.79 ± 7.91	57.71 ± 9.88	
Lymphocytes (%)	42.00 ± 7.59	39.02 ± 7.36	
Monocytes (%)	1.25 ± 0.44	1.15 ± 0.36	
Eosinophils (%)	1.21 ± 0.41	1.09 ± 0.28	
Platelets ($\times 10^{9}$/L)	246.90 ± 63.55	221.60± 58.67	
* Data represents the average of 24 participants ±SD † Data represents the average of 18 participants ±SD ‡ Statistically significant			

The effect of "BOTOX-HA" injections on laboratory parameters

Biochemical

We found that there was no significant difference between the control and "BOTOX-HA" study group (p-value > 0.05) in the ALT, AST, GGT, CRP, and CK-MB levels (Table 5). Yet, we revealed that there was a significant decrease in serum ALP and creatinine levels within the study group who received both BOTOX-HA injections compared with the control group (Figure 1A and B). We also found a significant increase in serum LDH level within the same study group compared with the control group (Figure 1F).

Immunological

Our results also showed that there was an increase in the TNF-α level in our "BOTOX-HA" study group. Nonetheless, it was statistically insignificant (p-value > 0.05) (Table 5).

TABLE 5. Effect of "BOTOX-HA" injections on biochemical and immunological parameters

Biochemical and immunological parameters			
Parameter	**Mean of control group** * **(U/L)**	**Mean of study group** † **(U/L)**	**P-value**
ALP	70.38 ± 20.56	58.86 ± 17.23	0.02 ‡
ALT	13.58 ± 6.51	18.29 ± 28.05	> 0.05
AST	18.58 ± 6.37	17.17 ± 6.90	
GGT	10.75 ± 10.38	13.29 ± 9.44	
Creatinine	0.87 ± 0.16	0.78 ± 0.17	0.01‡
CRP	2.65 ± 1.24	3.347 ± 2.78	> 0.05
LDH	316.90 ± 118.70	428.50 ± 168.70	0.02 ‡
CK-MB	13.42 ± 7.80	11.10 ± 5.20	> 0.05
TNF-α	9.19 ± 2.67	43.17 ± 113.30	
IL-17	6.39 ± 2.99	6.36 ± 3.77	
* Data represents the average of 24 participants ±SD † Data represents the average of 18 participants ±SD ‡ Statistically significant			

Hematological

As illustrated in in Table 6, there was no significant difference between both groups in all the hematological parameters (p- value >0.05).

TABLE 6. Effect of "BOTOX-HA" injections on Hematological parameters

Hematological parameters			
Parameter	**Mean of control group** *	**Mean of study group** †	**P-value**
Hb (g/dl)	13.49 ± 1.39	12.55 ± 1.75	> 0.05
RBCs ($\times 10^{12}$/L)	4.989 ± 0.44	4.979 ± 0.50	
RDW (%)	13.03 ± 0.79	13.33 ± 0.68	
WBCs ($\times 10^{9}$/L)	6.938 ± 1.50	6.075 ± 1.57	
Neutrophils (%)	56.79 ± 7.91	55.38 ± 16.05	
Lymphocytes (%)	42.00 ± 7.59	38.50 ± 13.55	
Monocytes (%)	1.250± 0.44	1.375 ± 0.52	
Eosinophils (%)	1.208± 0.41	1.000 ± 0.00	
Platelets ($\times 10^{9}$/L)	246.9 ± 63.55	206.4 ± 34.99	
* Data represents the average of 24 participants ±SD † Data represents the average of 18 participants ±SD			

Comparative analysis of biochemical, immunological, and hematological parameters between all study groups

Biochemical, immunological, and hematological parameters were also compared between the three study groups. Our findings revealed that there was no significant difference between "only BOTOX" vs "only filler", "only BOTOX" vs "BOTOX-HA", and "only filler" vs "BOTOX-HA" study groups in the biochemical (Figure 1), immunological (Figure 2) and hematological (Figure 3) parameters.

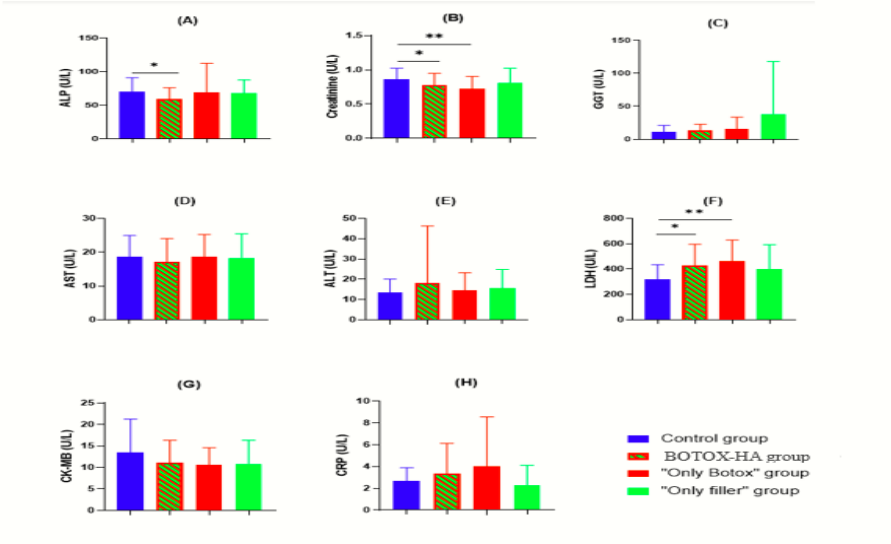

FIGURE 1. This figure represents a comparative analysis of the Biochemical parameters between the three study groups in all the fore-mentioned parameters (p-value > 0.05). Significant difference was only found in the ALP (A) level between control and "BOTOX-HA" study group (p-value < 0.05). Also, Creatinine (B) and LDH (F) levels were significantly different between the control vs "BOTOX-HA" and control vs "Only BOTOX" study groups.

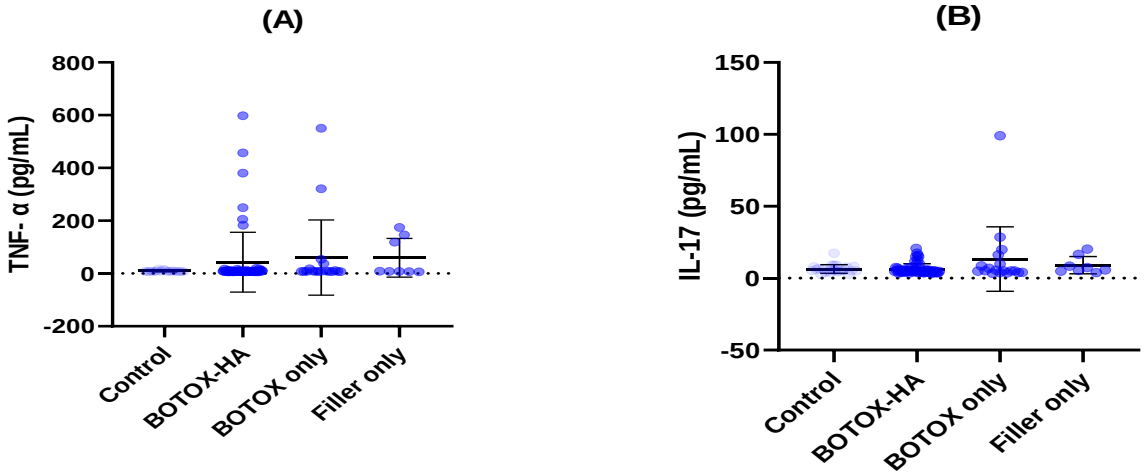

FIGURE 2. This figure represents a comparative analysis of the immunological parameters between the three study groups. (A) TNF-α, and (B) IL-17. Between the study groups, there was no significant difference in both parameters (P-value > 0.05).

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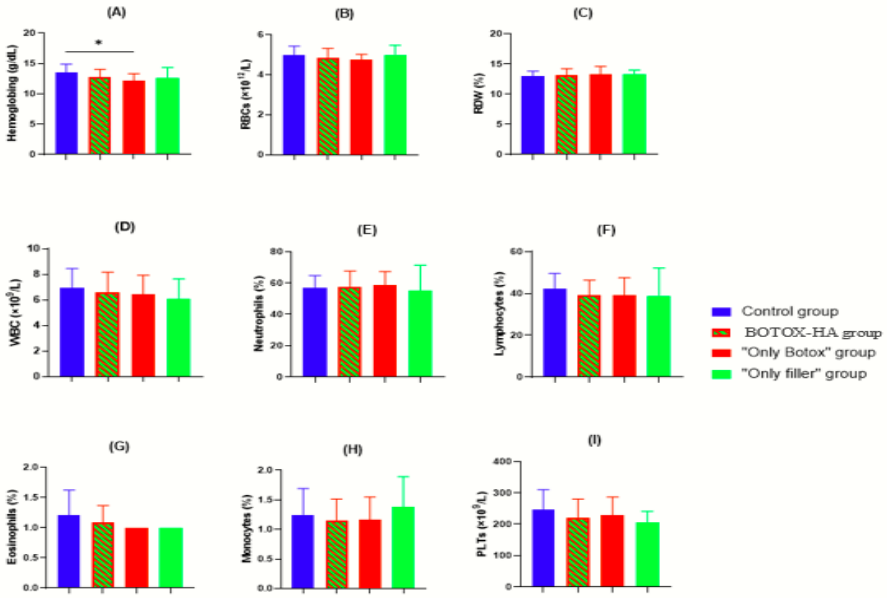

FIGURE 3. This figure represents a comparative analysis of the hematological parameters between the three study groups. (A) Hemoglobin, (B) RBCs (C) RDW (D) WBCs (E) Neutrophils (F) Lymphocytes (G) Eosinophils (H) Monocytes and (I) Platelets. No significant difference was found between the study groups in all the hematological parameters (p-value > 0.05). Significant difference was only found in the hemoglobin level between control and “BOTOX” study group (p-value < 0.05).

DISCUSSION

To the highest of our knowledge, this is the first study in Jordan to investigate how different serum biochemical and immunological parameters of healthy persons are affected by BOTOX and HA filler. Initially, in the current study, we examined the effect of BOTOX and HA filler on the activities of serum liver enzymes (ALT, AST, ALP, and GGT), cardiac enzymes (CK-MB), LDH, creatinine levels, inflammatory markers (IL-17, TNF α, CRP), and CBC.

This study found that liver enzymes did not significantly change after BOTOX injections, Although ALP demonstrated some reduction. Contrary to our results, when the effect of botulinum toxins on liver function in drinking water was evaluated, results showed an elevation in ALT and AST (23). Another study involving adult albino rats showed that BOTOX injections in the bones and bone marrow increased ALP. [24], while the present study showed a significant decrease in ALP in the group injected with BOTOX and HA filler. In line with our findings, an analysis performed in 2019 illustrated a reduction in the ALP level of mice injected with botulinum toxin (24).

The current study discovered that the HA filler had no discernible effect on liver enzymes. In line with this finding, a previously reported study indicated that HA application or injection did not negatively affect the liver (25). While another study showed that HA was found to promote the development of calcium oxalate crystals that adhere to the tubular rejoin of the kidney (26). In a recent study, HA microneedles were utilized to treat rheumatoid arthritis, and it was discovered that pro-inflammatory cytokines like IL-17 and TNF- were suppressed (27).

Besides that, both the "only BOTOX" and also the "BOTOX and HA filler" study group's creatinine levels significantly decreased, according to our results.

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Although the difference in the creatinine level between the control and treated individuals was statistically significant, this finding is probably biologically noteworthy since the creatinine in our study group was still within the normal range. This could be due to the different muscle mass between both groups. After all, studies have shown that muscle mass affects creatinine levels which could have been reflected in our creatinine results (28, 29).

One of the routinely performed methods for determining cytotoxicity is measuring the activity of many cytoplasmic enzymes released by injured cells. LDH is one of these enzymes that is found in all intact cells, and it is released from their damaged plasma membrane when cells undergo necrosis or apoptosis (26, 30). Until now, we have not found any published clinical studies that have examined the effects of BOTOX or HA filler injections on serum LDH levels in healthy individuals, so this study has the novelty of testing some new parameters. In our research, we found a statistically significant increase in LDH levels in both "only BOTOX" and "BOTOX and HA" study groups which may indicate the onset of necrosis at the injection site or systemic level. Whereas there was no significant effect of the "only filler" injections on our study subjects, this suggests that BOTOX injections, whether alone or combined with HA, could have a cytotoxic effect.

The liver produces C-reactive protein, an inflammatory protein that increases in response to inflammation (31). Our findings revealed an increase in CRP serum levels of both "only BOTOX" and "BOTOX-HA" study groups; although this increase was statistically insignificant, it may suggest that BOTOX injections do somehow induce an inflammatory response. Contrary to our findings, another study revealed no significant effect of BOTOX injection on CRP levels [25]. Nevertheless, these results might be confirmed with a bigger sample size with repeatedly injected subjects.

This study also evaluated the hematological effects of BOTOX injections, and we found a significant decline in Hb level in the group receiving "only BOTOX." In agreement with our findings, a recent investigation into the effect of BoNT/A injections on mice showed a significant decrease in RBC count, Hb level, and hematocrit (24). However, the reasons for this reduction in Hb level are not well understood, as it is well known that some medications may cause a decrease in Hb level (32), so the effect of BOTOX and HA injection needs further investigation.

The present study discovered that the insignificant increase in the IL-17 level could imply the absence of post-injection inflammatory response, which provides insight into the safety of BOTOX, HA filler, and the combination of both. Furthermore, other researchers found that serum IL-2, IL-6, and IL-8 levels, cytokines that promote the production of IL-17, were significantly higher in subjects who received BOTOX injections than in control (33).

While some results indicated that HA injections reduce important inflammatory markers such as IL-1, IL-8, reactive oxygen species levels, and IFN-γ (34), our results showed no significant effect of HA filler on IL-17. Sadly, only a few studies have investigated how BOTOX affects skin and muscle tissue after repeated injections (35). Another immunological parameter that was analyzed in this study is TNF-α. TNF- is a cytokine that is primarily produced by macrophages in response to inflammation, and it serves to warn other immune cells of the presence of inflammation (36). The present study denoted no significant effect of all treatments on TNF-α. The mean value of TNF-α in the control group (0-22 pg/mL) was within the normal range, while it was much higher in all treated groups (>40 pg/mL). Nevertheless, the difference in TNF-α was not statistically significant as most treated individuals had normal values, while few had extremely high concentrations. The extreme values resulted in huge variation between TNF-α concentrations within the treated groups, reducing the significance of the difference. At the same time, TNF-α serum levels were elevated in a closely related study that investigated the effect of BOTOX injection on inflammatory markers (37).

Also, it was reported that there was a massive increase in TNF-α after BOTOX injections, which may be due to a higher dose or repeated injections [38]. On the other hand, a more recent study that used HA microneedle to treat RA revealed that the levels of TNF-α were suppressed (27).

The current study revealed that using BOTOX and HA filler has a minor effect on biochemical tests and inflammatory markers. However, some of the liver enzymes and blood indices were affected, as we found a significant increase in LDH, a decrease in Cr and Hb in the "only BOTOX" group, and an increase in LDH and a reduction in Cr and ALP in the "BOTOX and HA filler" group compared with the control group.

These discrepancies may have been due to the difference in dose levels, expression patterns of the target genes, animal species used, and experiment duration (24). In addition, different time point measurements could contribute to the discrepancies in results.

CONCLUSION

Accordingly, using BOTOX and HA filler may result in mild changes in clinical laboratory tests. Additionally, it's crucial to investigate the effects of prolonged usage of these techniques and understand how they affect the body because most studies focus on side effects on the skin. Still, we need a lot of investigations regarding the body's tissues and whether they are damaged, as well as further clinical laboratory tests on a more significant number of samples to see if BOTOX or HA leads to changes in laboratory tests. Future investigations are now necessary to highlight the influence of these cosmetic procedures on various clinical laboratory parameters.

Ethical approval

Research involving human subjects was approved by the ethical committee at Al-Ahliyya Amman University-Deanship of Scientific Research University (reference number: IRB; AA-2-3-21). and comply with all relevant national regulations, institutional policies, and the ethics of the 2013 revision of the Helsinki Declaration.

Research funding

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Conflict of interest

Authors state no conflict of interest.

Informed consent

Every participant in this study gave their consent after receiving information about it.

Author contributions

Al-Karawi A. performed the statistical analysis and drafted the manuscript., Atoom A. contributed to the conception and design of the study, Al-Adwan S. was involved in clinical evaluation, Adwan S. interpreted the results and supervised the study, The entire manuscript was read and approved by all authors.

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