Pakistan J. Zool., pp 1-10, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220925230911

Aging and Infection-Based Alterations in Collagen Type 1 of Nile Tilapia (*Oreochromis niloticus* L.) Skin

Hossam I. Kadira[1]*, Ibrahim I. Al-Hawary[1], Abdallah Salah[2,3] and Zizy I. Elbialy[1]

[1]Department of Fish Processing and Biotechnology, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt.
[2]Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt.
[3]Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK.

Article Information
Received 25 August 2022
Revised 28 June 2023
Accepted 24 July 2023
Available online 25 April 2024
(early access)

Authors' Contribution
Conceptualization, methodology and writing: HIK and ZIE; Formal analysis and data curation: HIK and AS; Writing – review and editing: HIK, IIH, AS and ZIE; Supervision: IIA and ZIE.

Key words
Marine collagen, Nile tilapia, *col 1a1*, *col 1a2*, Collagen genes, Infection

ABSTRACT

Collagen is the amplest animal protein polymer and makes up about 30% of the total protein in the animal body. Collagen extracted from fish waste is not associated with serious infectious diseases nor is there religious objection. Here, we investigated the collagen genes relative expression (*col 1a2* and *col 1a1*) in the skin of Nile tilapia at 5, 7, 9 and 12 months of age as well as the effect of infection with *Aeromonas hydrophila* on regulating collagen genes to study the correlation between the transcription levels of collagen genes (*col 1a1* and *col 1a2)* and the immune-related genes (*IL-1 β, IFN- γ and TGF-β*) in order to demonstrate the association between them. Additionally, the immune-related genes regulation levels in control and infected groups (P<0.05) were tested to comprehend that association. The highest regulation levels of collagen genes had been noticed in 9 months age group. Noticeably the infection triggered a response which significantly reduced collagen genes expression that was indicated with the significant upregulation of both of *IL-1β, INF-γ*. However, *TGF-β* transcription was downregulated. For collagen industry and research purposes, collagen protein is isolated from 9 months age un-infected Nile tilapia fish is recommended for the best quality and quantity.

INTRODUCTION

In connective tissue from animal skin and bone, collagen is the dominant structural protein (Gelse *et al*., 2003; Singh *et al*., 2011). Importantly, collagen polymer comprises three polypeptide chains that form a triple helix. All collagens substantially differ in terms of their size, function, and tissue settlement (Gelse *et al*., 2003). Nearly 28 types of collagens, namely type I to XXVIII have been defined yet (Gordon and Hahn, 2010). Type I collagen is the amplest fibrillar collagen type in tetrapods widely distributed in bone tissue, cartilage, dentine, and plays a crucial role as structural support and as a biological signal to the surrounding cells. This extracellular matrix protein is encoded by the genes, *col 1a2* and *col 1a1* that express the α2 (I) and α1 (I) chain, respectively (Gistelinck *et al*., 2018).

The perpetual development of natural biomaterials from non-mammalian sources such as marine collagen could enhance the safety of its use in regenerative medicine, because collagen extracted from fish waste, contrary to bovine and poultry collagen, is not associated with serious zoonotic risks including bovine spongiform encephalopathy, foot and mouth disease, Ebola hemorrhagic fever, avian and swine influenza, and Zika fever that could be transmitted from other sources of collagen (Capati *et al*., 2016; Hayashi *et al*., 2012).

Collagen generated from both porcine and bovine sources cannot be used in some countries due to religious objections (e.g., both Islam and Judaism prohibit the eating of any pork-derived products, while Hindus do not eat any cow-derived products) (Huang *et al*., 2016). Thus, to minimize the potential transmission of zoonotic diseases and, in addition to the use of recombinant technology, marine collagen is a safe and cost-effective alternative source of type I collagen (Tang and Saito, 2015).

As the global demand for collagen has risen dramatically in recent years, fishes, which are considered the most genetically distant relatives in the lineage of

* Corresponding author: Hossam_ismaeel@fsh.kfs.edu.eg, hoskadira@gmail.com
0030-9923/2024/0001-0001 $ 9.00/0

mammals, have recently drawn attention as a promising source with low immunogenicity as well as getting an additional value for economic returns in the fishery industry by optimizing the use of fish wastes (Yamada *et al*., 2014).

Marine collagens were proposed as a feasible ingredient in the manufacturing process of functional food, cosmetics, biomedical as well as pharmaceutical applications. In particular, the most common sources of marine collagen were extracted from Nile tilapia skin (*Oreochromis niloticus*) (Dawood *et al*., 2020); sea cucumber (*Stichopus vastus*) (Abedin *et al*., 2013), pangasius catfish (*Pangasianodon hypophtalamus*) (Singh *et al*., 2011), mullet (*Mugil cephalis*), Atlantic flying fish species (*Cypselurus melanurus*), Synodontidae *(Saurida* spp.), jack mackerel (*Trachurus japonicus*); yellowback seabream (*Dentex tumifrons*) (Okazaki and Osako, 2014) and Atlantic salmon (*Salmo salar*) (Moreno Conde *et al*., 2012).

Nile tilapia (*Oreochromis niloticus*) is between the most economically valuable and widely cultured freshwater fish types, due to its superior meat quality, growing market demand, and well-established rearing protocols (Dawood *et al*., 2020). Tilapia is one of the most used fish in the filleting industry (Zarini, 2016).

In tilapia filleting, non-edible tissues such as skin, bones, and scales can contain up to 80% protein, which can trigger potential environmental concerns if not properly processed (Lin *et al*., 2017). Importantly, from these non-edibles, highly bioavailable marine collagen can be extracted.

Fish skin is considered a critical regulatory organ that acts not only as a physical barrier against pathogen invasion, but also as a complicated integrator of both environmental and nutritional clues through roles in osmoregulation, immunity, and endocrine signaling. The mucus layer, secreted by epidermal goblet cells, is essential for the physiological complexity of teleost skin (Li *et al*., 2013). In this context, collagen is considered the key extracellular protein involved in the skin defense mechanism. Because of the unique marine ecological ecosystem, such as low temperature, high salinity and high pressure, isolated marine collagen differs apparently from terrestrial sources collagen in both physicochemical characteristics and amino acid profiling (Hu *et al*., 2017), Furthermore, prominent physiological functions including antibacterial (Ennaas *et al*., 2016), antioxidant (Wang *et al*., 2008, 2013), anti-hypertensives (Kim *et al*., 2012), neuroprotective agent (Xu *et al*., 2015) and anti-skin aging activities.

It is widely reported that throughout aging, the dermal extracellular matrix is progressively degraded with alterations in the physiological function of resident fibroblast cells (Li *et al*., 2015). As fragmentation of collagen causes attachment sites loss, which results in a reduction of fibroblast distribution, which is recognized in elderly human skin. This contracted state, along with collateral affected mechanical force, is combined with the down-transcription of collagen synthesis in human skin (Fisher *et al*., 2008; Xia *et al*., 2013); therefore, the role of exogenous collagen has a promising role to counteract the effect of aging.

According to El Deen *et al*. (2014), *Aeromonas hydrophila* is one of the most opportunistic pathogens in fish which is commonly distributed in tropical regions (Egypt as well as other countries) aquaculture (both fresh and seawater), it has been indicated to trigger diseases in warm water fish especially Nile tilapia. Gestational infection with the zika virus suppresses the expression of collagen genes (*col 1a2, col 1a1, col 5a1, col 3a1, col 6a3, col 5a2, col 14a1,* and *col 12a1)* which are crucial for the formation of blood-brain barrier in newly born along with the downstream inhibition of proteins involved in collagen formation in (*col 1a1* and *col 1a2*) of patients with Zika virus congenital syndrome (Aguiar *et al*., 2019).

A new study (Cai *et al*., 2022) examined the transcriptome analysis of Atlantic salmon skin in response to the infectious salmon anemia Virus and sea lice (*Lepeophtheirus salmonis*) co-infection, collagen genes transcription (e.g., *col 10a1, col 11a1, col 12a1,* and *col 24a1*) were significantly downregulated in the co-infection compared to pre-infection.

Another study (Baumert *et al*., 2009) concluded that the capacity of fibroblasts to express both type I and type III collagen and fibronectin is diminished in response to infection with *Chlamydia pneumoniae*. These findings reveal that infected host cells principally release cytokines that employ restraining effects on the formation of matrix proteins. Thus, the current study's goal is to determine the best age for Nile tilapia collagen extraction based on the expression levels of the *col 1a1* and *col 1a2* genes and the effect of infection on collagen regulation in fish via the association of collagen type 1 and immune-related genes.

MATERIALS AND METHODS

Sample collection

Forty monosex male Nile Tilapia fish (*Oreochromis niloticus*), at age stages intervals of 5, 7, 9 and 12 months (10 fish for each age stage) with average body weight of 58±16g, 92±2.4g, 162±6.2g and 234±7.89, respectively from the same fish population from a private farm in Kafrelsheikh, Egypt, were transported alive for adaption to laboratory conditions for 2 weeks for further collection

of skin samples.

In a separate container, fish were euthanized with an overdose of buffered MS-222 (200 mg/L MS-222 + 400 mg/L sodium bicarbonate), Skin samples were collected from all fish using a sterile scalpel and placed in sterile 2mL-Eppendorf tubes before being shocked in liquid nitrogen and stored at -80 ºC until further gene expression analysis. For challenge trial, a PCR- identified pathogenic *Aeromonas hydrophila* strain obtained from the Department of Fish Diseases of the University was used for induction of fish infection. Sixty healthy Nile tilapia (aged 9 months) were obtained from the farm and were acclimated to the laboratory conditions, then divided into two groups, distributed in 6 glass aquaria (80×50×40 cm) with three replicates for each group. Each tank was filled with 130 liters of dechlorinated tap water and stocked with 10 fish (164 ± 6.1g). Fish in both groups were fed a basal diet of 3% of their body weight twice daily.

Fish were anaesthetized with MS-222 ® (Sigma-Aldrich®, USA) prior to the challenge, and the scales were gently scraped off with a scalpel between the caudal peduncle and the pectoral fin, as described by (Adikesavalu *et al*., 2015). The abraded fish were then immersed in a 1000ml suspension containing *A. hydrophila* at dose of 2×10^7 CFU/mL for 1 h. Furthermore, the abraded fish of the control group were dipped in 0.85% saline instead of bacterial suspension for 1 h. Seven days post-challenge, the control and the challenged group were sampled (10 fish /group) to examine the differential expression of the studied genes in the skin after anaesthetization with MS-222 ® (Sigma-Aldrich®, USA), then samples were kept in sterile 2mL- Eppendorf tubes before snap shocking in liquid nitrogen tank and stored at – 80 °c for subsequent total RNA extraction.

Total RNA extraction and cDNA synthesis

TRIzol™ (iNtRON Biotechnology, Inc., South Korea) was used to extract total RNA from 100 mg of skin tissue according to the manufacturer's instructions. A nanodrop spectrophotometer (BioDrop®, USA) was used to ensure the quantity and quality of the extracted RNA, and RNA samples were run on an agarose gel stained with ethidium bromide (EtBr) to ensure their integrity. The presence of sharp, high-intensity bands of 28S and 18S rRNA was thought to indicate integrity, with the 28S rRNA band being roughly twice as intense as the 18S rRNA band. 2 L of extracted RNA was reverse transcribed into cDNA using the Maxime™ RT PreMix Kit (iNtRON Biotechnology, Inc., South Korea) according to the manufacturer's instructions.

Primer design

Tilapia collagen type 1 gene primers (*col 1a1* and *col 1a2*) were designed using the primer-BLAST online tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), while other primer sequences (*IL-1β, IFN-γ, TGF-β*, *18s rRNA* and *β-actin*) were reviewed from previous literatures, as shown in Table I. Internal control reference genes -actin and 18s rRNA were used.

Table I. Sequence of tilapia primers used for qPCR analysis.

Gene*	Primer sequence 5′–3′	Annealing temperature (°C)	Amplicon size (bp)	Amplification efficiency (%)	NCBI gene bank accession no.	Reference
col 1a1	F: CAGCCTGGAGTCATGGGATT R: CGCCAACATCACCATCCTTTC	57	127	0.98	NM_001279444.1	This study
col 1a2	F: ACCCAATGGATACAGCGGAC R: GGCTGGTCAGCTCTGTACTC	56	123	0.99	NM_001282897.1	This study
IL-1 β	F: CAAGGATGACGACAAGCCAACC R: AGCGGACAGACATGAGAGTGC	61	149	0.81	XM_003460625.2	(Elbialy *et al*., 2021)
IFN- γ	F: AAGAATCGCAGCTCTGCACCAT R: GTGTCGTATTGCTGTGGCTTCC	61	116	0.84	XM_005448319.1	(Qiang *et al*., 2016)
TGF-β	F: GTTTGAACTTCGGCGGTACTG R: TCCTGCTCATAGTCCCAGAGA	60	81	0.88	XM_003459454.2	(Standen, 2015)
β-actin	F: CCACACAGTGCCCATCTACGA R: CCACGCTCTGTCAGGATCTTCA	63	217	0.98	XM_003455949.1	(Yang *et al*., 2013)
18 s rRNA	F: GGACACGGAAAGGATTGACAG R: GTTCGTTATCGGAATTAACCAGAC	65	111	0.92	JF698683	(Yang *et al*., 2013)

col 1a1, collagen type 1 alpha 1; *col 1a2*, collagen type 1 alpha 2; *IL-1β*, Interleukin 1 beta; *IFN- γ*, Interferon gamma; *TGF β*, transforming growth factor beta.

Relative gene expression analysis

The Mic-qPCR thermocycler (Bio-molecular systems, USA) was used to profile gene expression in a 25-µl reaction volume. The qPCR mixture contains 12.5 µl of 2× SYBR Green/no-ROX rtPCR master mix (SensiFAST cDNA kit, Bioline, UK), 2 µl of cDNA template, 1 µl of forward primer, 1 µl of reverse primer, and 8.5 µl of nuclease-free water.

qPCR reaction was directed according to the following protocol: preliminary denaturation at 95°C for 10 min, followed by 40 cycles: 15 s at 95°C, 35 s at the specific annealing temperature as described in table (2), and extension for 20 s at 72 °C. To ensure primer specificity, melting curve of 0.5°C increment in temperature range from 65-95°C was finished with the end of qPCR reaction. Amplification efficiency was calculated based on the qPCR slope via the formula: $E = -1+10^{(-1/slope)}$ (Rasmussen, 2001). QPCR data normalization was performed using the geometric averaging of the two reference genes used in this study *18 s rRNA* and *β-actin* genes for accurate calculation of fold change.

Relative expression of collagen type 1 genes and at different Nile tilapia age stages was calculated according to the -ΔCt method according to (Yuan *et al.*, 2006). However, relative expression of collagen type 1 genes and immune-related genes in challenge trial were normalized against the control group using the $2^{-\Delta\Delta Ct}$ method according to (Pfaffl, 2001).

Statistical analysis

All data are presented as Means ± SEM. The statistical significance difference of the relative expression at different Nile tilapia ages were estimated by One-way analysis of variance (ANOVA) test tracked by Tukey's multiple comparison as post-hoc test using (GraphPad Software, San Diego, California USA) version 8.00, however the statistical significance difference of the Relative expression of collagen type 1 genes and immune-related genes in challenge trial was evaluated using t-test (unpaired t-test). The correlation between collagen type 1 genes and immune-related genes post-infection was evaluated by Pearson correlation matrix analysis. Differences were considered as significant at $p \leq 0.05$.

RESULTS

Collagen type 1 genes regulation at different Nile tilapia ages

Importantly, *col 1a1* showed the highest regulation level at 9 months age compared to the other ages, while the lowest level was observed in 7 months age. Synchronously, similar finding was noticed for *col 1a2*. The expression levels between 9 months and the other age stages in both (*col 1a1* and *col 1a2)* are statistically significant at $p<0.05$ (Fig. 1).

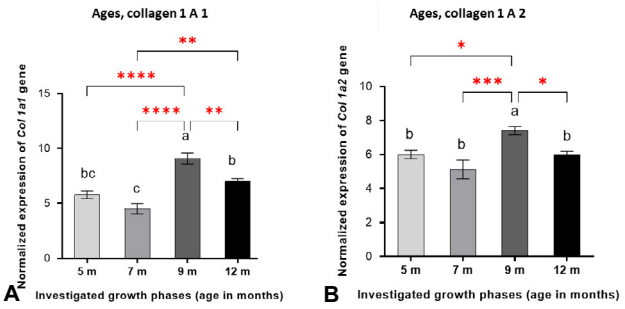

Fig. 1. Relative expression of collagen type 1 genes at different Nile tilapia age stages skin. A) relative expression of *col 1a1* gene B) relative expression of *col 1a2* gene. Groups with different asterisks indicate a significance difference between them, (* when $p<0.05$), (** when $p<0.01$), (*** when $p<0.001$)

In challenge trial, as concluded in Figure 2, bacterial infection triggered a response which significantly reduced the expression of both *col 1a1 at (p> 0.001)* and *col 1a2 at (p > 0.01)* genes between the control and the infected groups. As indicated in Figure 3, both of *IL-1β at (p> 0.05), INF-γ at (p> 0.01)* regulation significantly upregulated between the control and infected groups, noteworthy *TGF-β* transcription downregulated, however the differences are non-significant.

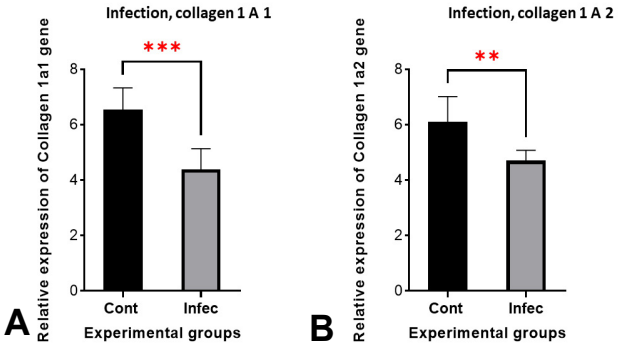

Fig. 2. Relative expression of collagen type 1 genes in response to *Aeromonas hydrophila* infection. (A) *col 1a1* expression in both the control and the infected group. (B) *col 1a2* expression in both the control and the infected group. (** when $p<0.01$), (*** when $p<0.001$).

In infection trial, Significantly the regulation levels of both *col 1a2* and *col 1a1* is positively associated with each other (correlation coefficient =0.84). Both of

col 1a2 and *col 1a1* transcription levels were inversely correlated to both *IL-1 β* and *IFN- γ* genes, despite of their directional proportion to *TGF-β* expression. Importantly, *IL-1 β* expression pattern revealed an inverse correlation with *TGF-β*. The stated correlation between *col 1a1* and immune-related genes were significantly different (p<0.01), as well as between *col 1a2* and immune-related genes (P<0.05) according to asterisk demonstrated by (Fig. 4).

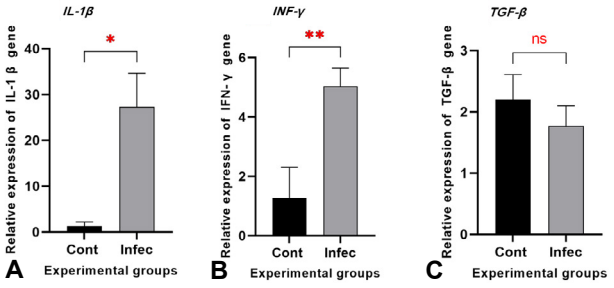

Fig. 3. Relative expression of immune-related genes in response to *Aeromonas hydrophila* infection in both the control and the infected group. (A) *IL-1β* expression. (B) *INF-γ* expression. (C) *TGF-β* expression. Significance difference between *Aeromonas hydrophila* infection and control is indicated with asterisks. *(p>. 0.05*) for 1 asterisk, while *(p> 0.01)* for 2 asterisks.

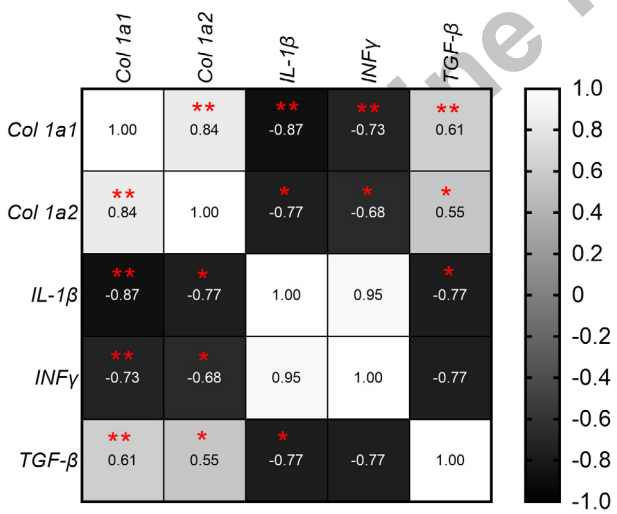

Fig. 4. Heat map demonstrating the correlation matrix of the relative expression of studied genes in post-infected tilapia fish (9 months age). Correlation coefficient is texted in each cell and labeled with significance asterisks, the superscript (*) indicates that the correlation is significant at p. value ≤ 0.05, whereas (**) indicates that it is significant at p. value ≤ 0.01

DISCUSSION

Collagen is between the most abundant proteins in animal kingdom. However, it is not existing in plants as well as unicellular creatures as polysaccharides along with cellulose achieve its role (Silvipriya *et al*., 2015). Collagen provides protect the skin by delaying absorption of both pathogens and toxins (Fratzl, 2008). Collagens play an important role in cellular biological functions such as cell survival, proliferation, and differentiation. They also aid in the healing of damaged bones or blood vessels and protect tissue structural integrity (Buehler, 2006).

Several age-related modifications have been discovered in skin fibroblast cells in vivo that may be responsible for a decrease in collagen production in the form of collagen fibril fragmentation (Li *et al*., 2015).

In our study, collagen was highly and significantly expressed at 9 months of age, where the Nile tilapia is mature, and started to decline at 12 months of age. Our results are in consistence with those of Takatsu *et al*. (1999) who stated that the mRNA level of collagen type I, which is one of the major components of collagen fibers in ligaments, declined with age that could be assigned to increased methylation sites of collagen genes. They explained their as deacetylation of histones located at the positions where DNA is methylated could be involved in suppression of gene transcription via making the chromatin structure unavailable to transcription factors (Nan *et al*., 1998).

In the same context, Fisher *et al*. (2008) hypothesized that old fibroblast cells have an age-dependent decrease in their capacity for collagen synthesis and jointly experience a loss of mechanical stimulation that results from diminished intact collagen fibers.

The same results were demonstrated by Podolsky *et al*. (2020) who demonstrated that collagen turnover decreased over the life span of tested mice, and this decrease is characterized by decreased collagen transcription, cell-mediated collagen internalization, and MRC2 degradation (mannose receptor, C-type 2), which agree with our results regarding age-related decline in collagen expression.

Our findings revealed that collagen type I genes, as a major constituent of the extracellular matrix is orchestrated with immune response in bacterial infection.

These results are in agreement with Aguiar *et al*. (2019) who claimed that low levels of ECM proteins (fibronectin and collagen IV) increased the permeability of the blood-brain barrier, allowing ZIKV to reach developing neural progenitor cells and disrupt neural development in infected children. Infection's effect on ECM could result in cell death and dysregulation of ECM expression, or even tissue deposition as the case in our study where infection

with *A. hydrophila* resulted insignificant decrease in *col 1a1* and *col 1a2* regulation levels compared to control non-infected groups. Concurrently, previous studies according to Bateman *et al.* (2009) indicated that mutations in collagen type I can affect the extracellular matrix by reducing the amount of produced collagens, which can impair molecular and supramolecular assembly via mutant collagen production or induction of endoplasmic reticulum stress, as well as the unfolded protein response. Our findings are also in line with Baumert *et al.* (2009) who indicated that the capacity of fibroblast cells to express both type I and III collagen and fibronectin is diminished in response to infection with *C. pneumonia.*

IL-1β is an important pro-inflammatory cytokine which play a major role in the bacterial infection early responses by encouraging phagocytosis, lymphocyte activation, apoptosis, and leucocytes migration (Reyes-Cerpa *et al.*, 2012). Infection studies with *Gyrodactylus salaris* compared *IL-1β* regulation in the skin of infected and healthy salmon (Lindenstrøm *et al.*, 2006). According to our findings, the infected salmon population had significantly higher levels of *IL-1β* to boost mucus secretion and thereby to enhance the possibilities for infection propagation, however the less infected population reported no significant increase (Lindenstrøm *et al.*, 2006), additionally, after 7 days of amoebic gill disease infection, *IL-1β1* in the gills and the liver of rainbow trout significantly upregulated (Bridle *et al.*, 2006). *IL-1β* late up-regulation was also stated in Atlantic Cod at (7–60) days post-infection, and the authors propose that transcription of this gene initially depend on the differences in the challenge dosages (Ellingsen *et al.*, 2011), which support the production of *IL-1β1* until 7 days post infection in different fish organs.

IFN-γ is one of the chief cytokines that plays a crucial role against intracellular bacterial infection (Swain *et al.*, 2015). Our result is in consistence to IFN-γ elevated level in the skin of Atlantic cod which have uncovered the significance of these molecules, even in the quiescent state of the host cells which has a vital role in macrophages activation for expanded killing of pathogens (Caipang *et al.*, 2011).

TGF-β is an eminent suppressive cytokine that provides immune tolerance and controls autoimmunity (Reyes-Cerpa *et al.*, 2012). *TGF-β* did not follow the common transcriptional change patterns observed in the other genes studied (Severin and El-Matbouli, 2007) has been reported that *TGF-β* revealed changes in the expression level which slightly varied from other cytokines included *IL-1β1*. *TGF-β* pathway could be repressed by *IL-1β* was confirmed by (Benus *et al.*, 2005), which agrees with *TGF-β* non-significance down-regulation in this study. *TGF-β* might be pro- or anti-inflammatory in line with its concentration, target cells differentiation state, and the concentration of other pro-inflammatory compounds (Severin and El-Matbouli, 2007).

Infection with *Mycobacterium marinum* in striped bass revealed intense inflammatory reactions in collaboration with *TGF-β* transcription down-regulation (Harms *et al.*, 2003), however, infected tilapia showed less inflammation and no more significant changes in *TGF-β* transcription, which is consistent with our findings. It is suggested that in fish that are sensitive to mycobacteria infection, *TGF-β* down-regulation may play a role in the down-regulation of inflammatory reactions that lead to extensive organ damage.

Col 1a1 regulation level is positively associated to thus of *col 1a2* which is in consistence with Gistelinck *et al.* (2018), which showed that collagen type 1 is the main constituent of the extracellular matrix protein which is encoded by both *col 1a2* and *col 1a1* genes through the regulation of both the α1 and α2 chains, respectively. Besides Thiele *et al.* (2004) indicated that *hnRNP-E* is a common mRNA-binding protein factor, which has a key role in type I collagen two subunits (*col 1a2* and *col 1a1)* mRNA stabilization.

Significantly, the transcription levels of both of *col 1a2* and *col 1a1* post-infection skin cells are positively correlated to *TGF β* which is in accordance with Ghosh *et al.* (2007), who demonstrated that *TGF-β* signals modulate the transcription levels of collagen Type I genes through *Smad* pathway stimulation in the skin fibroblasts, and maintain wound healing by stimulating fibrosis and extracellular matrix formation by fibroblasts (Reyes-Cerpa *et al.*, 2012).

The overall findings reveal that infected host cells principally release inflammatory *IL 1β* and *IFN γ* cytokines that employ inhibitory effects on matrix protein and fibronectin synthesis

Infection-associated *IL 1β* signals downregulated the transcription levels of both *col 1a2* and *col 1a1* (negative correlations) which is explained by Mia *et al.* (2014) *IL 1β* provokes the expression level of collagenolytic *MMP1* enzyme which has a potent anti-fibrotic effect.

IFN γ upregulation decreases fibroblast proliferation and type I collagen production primarily through destabilization of collagen type 1 mRNA (Bou-Gharios *et al.*, 2020), which emphasis the negative association between them in this work.

TGF β regulatory properties are primarily suppressive, including antagonism of pro-inflammatory cytokines such as IL-1β, activation of NK cells, and inhibition of macrophage activation (Severin and El-Matbouli, 2007), which explain the negative association between both *IL 1β* and *TGF β* genes.

Despite the current study's small sample size due to the lack of fund, our results gave an insight about the importance of ECM proteins (collagen type I) in tilapia different age stages and its association with immune-activity, so further studies on the other types of collagen genes and chemical analysis of collagen protein will be intended.

CONCLUSION

This work is the first reported study to investigate the impact of age progression and bacterial infection on the expression pattern of collagen type I genes in Nile tilapia fish (*Oreochromis niloticus*) and to describe the correlation between immune-related genes and ECM proteins (*col 1a1*- and *col 1a2*) in skin tissue exposed to bacterial infection**.** based on those findings, it is recommended to extract ECM proteins (collagen type I) from 9 months age healthy (non-infected) Nile tilapia skin for different research or/and bioindustries.

ACKNOWLEDGEMENT

The authors wish to acknowledge the support of the Biotechnology Lab., Faculty of Aquatic and Fisheries Science, Kafrelsheikh University, Egypt, where the laboratory investigations were carried out.

Data availability statement

The paper includes all of the data that supports the results or analyses.

Funding

No funding organization provided financial support for this study.

Ethical approval

The Ethical Committee of the Institutional Animal Care and Animal Ethics Committee, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, approved all animal treatments and experimental procedures (approval number: IAACUC-KSU-41-2020).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abedin, M.Z., Karim, A.A., Ahmed, F., Latiff, A.A., Gan, C.Y., Che Ghazali, F. and Islam, S.M.Z., 2013. Isolation and characterization of pepsin-solubilized collagen from the integument of sea cucumber (*Stichopus vastus*). *J. Sci. Fd. Agric.,* **93:** 1083-1088. https://doi.org/10.1002/jsfa.5854

Adikesavalu, H., Patra, A., Banerjee, S., Sarkar, A. and Abraham, T.J., 2015. Phenotypic and molecular characterization and pathology of *Flectobacillus roseus* causing flectobacillosis in captive held carp *Labeo rohita* (Ham.) fingerlings. *Aquaculture,* **439:** 60-65. https://doi.org/10.1016/j.aquaculture.2014.12.036

Aguiar, R.S., Pohl, F., Morais, G.L., Nogueira, F.C., Carvalho, J.B., Guida, L., Arge, L.W., Melo, A., Moreira, M.E. and Cunha, D.P., 2019. The potential role of collagens in congenital Zika syndrome: A systems biology approach. *bioRxiv***, 541268**. https://doi.org/10.1101/541268

Bateman, J.F., Boot-Handford, R.P. and Lamande, S.R., 2009. Genetic diseases of connective tissues: Cellular and extracellular effects of ECM mutations. *Nat. Rev. Genet.,* **10**: 173-183. https://doi.org/10.1038/nrg2520

Baumert, J.R., Schmidt, K.H., Eitner, A., Straube, E. and Rödel, J.R., 2009. Host cell cytokines induced by *Chlamydia pneumoniae* decrease the expression of interstitial collagens and fibronectin in fibroblasts. *Infect. Immun.,* **77:** 867-876. https://doi.org/10.1128/IAI.00566-08

Benus, G.F., Wierenga, A.T., De Gorter, D.J., Schuringa, J.J., Van Bennekum, A.M., Drenth-Diephuis, L., Vellenga, E. and Eggen, B.J., 2005. Inhibition of the transforming growth factor β (TGFβ) pathway by interleukin-1β is mediated through TGFβ-activated kinase 1 phosphorylation of SMAD3. *Mol. Biol. Cell,* **16:** 3501-3510. https://doi.org/10.1091/mbc.e04-11-1033

Bou-Gharios, G., Abraham, D. and De Crombrugghe, B. 2020. Type I collagen structure, synthesis, and regulation. In: *Principles of bone biology.* Elsevier. https://doi.org/10.1016/B978-0-12-814841-9.00013-0

Bridle, A.R., Morrison, R.N. and Nowak, B.F., 2006. The expression of immune-regulatory genes in rainbow trout, *Oncorhynchus mykiss*, during amoebic gill disease (AGD). *Fish Shellfish Immunol.,* **20:** 346-364. https://doi.org/10.1016/j.fsi.2005.05.014

Buehler, M.J., 2006. Nature designs tough collagen: Explaining the nanostructure of collagen fibrils. *Proc. natl. Acad. Sci.,* **103:** 12285-12290. https://doi.org/10.1073/pnas.0603216103

Cai, W., Kumar, S., Navaneethaiyer, U., Caballero-Solares, A., Carvalho, L.A., Whyte, S.K., Purcell, S.L., Gagne, N., Hori, T.S. and Allen, M., 2022. Transcriptome analysis of atlantic salmon (*Salmo*

salar) skin in response to sea lice and infectious salmon anemia virus co-infection under different experimental functional diets. *Front. Immunol.,* **5535**. https://doi.org/10.3389/fimmu.2021.787033

Caipang, C.M.A., Lazado, C.C., Brinchmann, M.F., Rombout, J.H. and Kiron, V., 2011. Differential expression of immune and stress genes in the skin of Atlantic cod (*Gadus morhua*). *Comp. Biochem. Physiol. D Genom. Proteom.,* **6:** 158-162. https://doi.org/10.1016/j.cbd.2011.01.001

Capati, M.L.F., Nakazono, A., Yamamoto, K., Sugimoto, K., Yanagiguchi, K., Yamada, S. and Hayashi, Y., 2016. Fish collagen promotes the expression of genes related to osteoblastic activity. *Int. J. Polymer Sci.,* **2016**. https://doi.org/10.1155/2016/5785819

Dawood, M.A., Moustafa, E.M., Elbialy, Z.I., Farrag, F., Lolo, E.E., Abdel-Daim, H.A., Abdel-Daim, M.M. and Van Doan, H., 2020. *Lactobacillus plantarum* L-137 and/or β-glucan impacted the histopathological, antioxidant, immune-related genes and resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*. *Res. Vet. Sci.,* **130:** 212-221. https://doi.org/10.1016/j.rvsc.2020.03.019

El-Deen, A.N., Dorgham-Sohad, M., Hassan-Azza, H. and Hakim, A., 2014. Studies on *Aeromonas hydrophila* in cultured *Oreochromis niloticus* at Kafr El Sheikh Governorate, Egypt with reference to histopathological alterations in some vital organs. *World J. Fish Mar. Sci.,* **6:** 233-240.

Elbialy, Z.I., Salah, A.S., Elsheshtawy, A., Rizk, M., Abualreesh, M.H., Abdel-Daim, M.M., Salem, S.M., Askary, A.E. and Assar, D.H., 2021. Exploring the multimodal role of yucca schidigera extract in protection against chronic ammonia exposure targeting: Growth, metabolic, stress and inflammatory responses in nile tilapia (*Oreochromis niloticus* L.). *Animals,* **11:** 2072. https://doi.org/10.3390/ani11072072

Ellingsen, T., Inami, M., Gjessing, M.C., Van Nieuwenhove, K., Larsen, R., Seppola, M., Lund, V. and Schrøder, M.B., 2011. *Francisella noatunensis* in Atlantic cod (*Gadus morhua* L.); waterborne transmission and immune responses. *Fish Shellfish Immunol.,* **31:** 326-333. https://doi.org/10.1016/j.fsi.2011.05.021

Ennaas, N., Hammami, R., Gomaa, A., Bedard, F., Biron, É., Subirade, M., Beaulieu, L. and Fliss, I., 2016. Collagencin, an antibacterial peptide from fish collagen: Activity, structure and interaction dynamics with membrane. *Biochem. Biophys. Res. Commun.,* **473:** 642-647. https://doi.org/10.1016/j.bbrc.2016.03.121

Fisher, G.J., Varani, J. and Voorhees, J.J., 2008. Looking older: Fibroblast collapse and therapeutic implications. *Arch. Dermatol.,* **144:** 666-672. https://doi.org/10.1001/archderm.144.5.666

Fratzl, P., 2008. *Collagen: Structure and mechanics, an introduction*. Collagen. Springer. https://doi.org/10.1007/978-0-387-73906-9

Gelse, K., Pöschl, E. and Aigner, T., 2003. Collagens structure, function, and biosynthesis. *Adv. Drug Delivery Rev.,* **55:** 1531-1546. https://doi.org/10.1016/j.addr.2003.08.002

Ghosh, A.K., Mori, Y., Dowling, E. and Varga, J., 2007. Trichostatin A blocks TGF-β-induced collagen gene expression in skin fibroblasts: Involvement of Sp1. *Biochem. biophys. Res. Commun.,* **354:** 420-426. https://doi.org/10.1016/j.bbrc.2006.12.204

Gistelinck, C., Kwon, R.Y., Malfait, F., Symoens, S., Harris, M.P., Henke, K., Hawkins, M.B., Fisher, S., Sips, P. and Guillemyn, B., 2018. Zebrafish type I collagen mutants faithfully recapitulate human type I collagenopathies. *Proc. natl. Acad. Sci.,* **115:** E8037-E8046. https://doi.org/10.1073/pnas.1722200115

Gordon, M.K. and Hahn, R.A., 2010. Collagens. *Cell Tissue Res.,* **339:** 247-257. https://doi.org/10.1007/s00441-009-0844-4

Harms, C.A., Howard, K.E., Wolf, J.C., Smith, S.A. and Kennedy-Stoskopf, S., 2003. Transforming growth factor-β response to mycobacterial infection in striped bass *Morone saxatilis* and hybrid tilapia *Oreochromis* spp. *Vet. Immunol. Immunopathol.,* **95:** 155-163. https://doi.org/10.1016/S0165-2427(03)00138-7

Hayashi, Y., Yamada, S., Guchi, K.Y., Koyama, Z. and Ikeda, T., 2012. Chitosan and fish collagen as biomaterials for regenerative medicine. *Adv. Fd. Nutr. Res.,* **65:** 107-120. https://doi.org/10.1016/B978-0-12-416003-3.00006-8

Hu, Z., Yang, P., Zhou, C., Li, S. and Hong, P., 2017. Marine collagen peptides from the skin of Nile tilapia (*Oreochromis niloticus*): Characterization and wound healing evaluation. *Mar. Drugs,* **15:** 102. https://doi.org/10.3390/md15040102

Huang, C.Y., Kuo, J.M., Wu, S.J. and Tsai, H.T., 2016. Isolation and characterization of fish scale collagen from tilapia (*Oreochromis* sp.) by a novel extrusion hydro extraction process. *Fd. Chem.,* **190:** 997-1006. https://doi.org/10.1016/j.foodchem.2015.06.066

Kim, S.K., Ngo, D.H. and Vo, T.S., 2012. Marine fish-derived bioactive peptides as potential

antihypertensive agents. *Adv. Fd. Nutr. Res.,* **65:** 249-260. https://doi.org/10.1016/B978-0-12-416003-3.00016-0

Li, C., Beck, B., Su, B., Terhune, J. and Peatman, E., 2013. Early mucosal responses in blue catfish (*Ictalurus furcatus*) skin to *Aeromonas hydrophila* infection. *Fish Shellfish Immunol.,* **34:** 920-928. https://doi.org/10.1016/j.fsi.2013.01.002

Li, Y., Lei, D., Swindell, W.R., Xia, W., Weng, S., Fu, J., Worthen, C.A., Okubo, T., Johnston, A. and Gudjonsson, J.E., 2015. Age-associated increase in skin fibroblast–derived prostaglandin E2 contributes to reduced collagen levels in elderly human skin. *J. Invest. Dermatol.,* **135:** 2181-2188. https://doi.org/10.1038/jid.2015.157

Lin, H.C., Alashi, A.M., Aluko, R.E., Sun Pan, B. and Chang, Y.W., 2017. Antihypertensive properties of tilapia (*Oreochromis* spp.) frame and skin enzymatic protein hydrolysates. *Fd. Nutr. Res.,* **61:** 1391666. https://doi.org/10.1080/16546628.2017.1391666

Lindenstrøm, T., Sigh, J., Dalgaard, M. and Buchmann, K., 2006. Skin expression of IL-1β in East Atlantic salmon, *Salmo salar* L., highly susceptible to *Gyrodactylus salaris* infection is enhanced compared to a low susceptibility Baltic stock. *J. Fish Dis.,* **29:** 123-128. https://doi.org/10.1111/j.1365-2761.2006.00696.x

Mia, M.M., Boersema, M. and Bank, R.A., 2014. Interleukin-1β attenuates myofibroblast formation and extracellular matrix production in dermal and lung fibroblasts exposed to transforming growth factor-β1. *PLoS One,* **9:** e91559. https://doi.org/10.1371/journal.pone.0091559

Moreno, C.H.M., Montero, G.P., Guillen, G., Fernández, M.F., Mørkøre, T. and Borderías, A.J., 2012. *Collagen characteristics of farmed Atlantic salmon with firm and soft fillet texture.* https://doi.org/10.1016/j.foodchem.2012.02.160

Nan, X., Ng, H.H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N. and Bird, A., 1998. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature,* **393:** 386-389. https://doi.org/10.1038/30764

Okazaki, E. and Osako, K., 2014. Isolation and characterization of acid-soluble collagen from the scales of marine fishes from Japan and Vietnam. *Fd. Chem.,* **149:** 264-270. https://doi.org/10.1016/j.foodchem.2013.10.094

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT–PCR. *Nucl. Acids Res.,* **29:** e45-e45. https://doi.org/10.1093/nar/29.9.e45

Podolsky, M.J., Yang, C.D., Valenzuela, C.L., Datta, R., Huang, S.K., Nishimura, S.L., Dallas, S.L., Wolters, P.J., Le Saux, C.J. and Atabai, K., 2020. Age-dependent regulation of cell-mediated collagen turnover. *JCI Insight,* **5**. https://doi.org/10.1172/jci.insight.137519

Qiang, J., He, J., Yang, H., Xu, P., Habte-Tsion, H.M., Ma, X. and Zhu, Z., 2016. The changes in cortisol and expression of immune genes of GIFT tilapia *Oreochromis niloticus* (L.) at different rearing densities under Streptococcus iniae infection. *Aquacult. Int.,* **24:** 1365-1378. https://doi.org/10.1007/s10499-016-9995-y

Rasmussen, R., 2001. *Quantification on the light cycler. Rapid cycle real-time PCR.* Springer. https://doi.org/10.1007/978-3-642-59524-0_3

Reyes-Cerpa, S., Maisey, K., Reyes-López, F., Toro-Ascuy, D., Sandino, A.M. and Imarai, M., 2012. Fish cytokines and immune response. *New Adv. Contrib. Fish Biol.*, pp. 3-57. https://doi.org/10.5772/53504

Severin, V.I. and El-Matbouli, M., 2007. Relative quantification of immune-regulatory genes in two rainbow trout strains, *Oncorhynchus mykiss*, after exposure to *Myxobolus cerebralis*, the causative agent of whirling disease. *Parasitol. Res.,* **101:** 1019-1027. https://doi.org/10.1007/s00436-007-0582-z

Silvipriya, K., Kumar, K.K., Bhat, A., Kumar, B.D., John, A. and Lakshmanan, P., 2015. Collagen: Animal sources and biomedical application. *J. appl. Pharm. Sci.,* **5:** 123-127. https://doi.org/10.7324/JAPS.2015.50322

Singh, P., Benjakul, S., Maqsood, S. and Kishimura, H., 2011. Isolation and characterisation of collagen extracted from the skin of striped catfish (*Pangasianodon hypophthalmus*). *Fd. Chem.,* **124:** 97-105. https://doi.org/10.1016/j.foodchem.2010.05.111

Standen, B., 2015. *The effect of dietary probiotics on Nile tilapia,* Oreochromis niloticus*, health and growth performance*. PhD thesis, Faculty of Science and Environment, Plymouth University, UK. https://dx.doi.org/10.24382/3312

Swain, B., Basu, M., Lenka, S.S., Das, S., Jayasankar, P. and Samanta, M., 2015. Characterization and inductive expression analysis of interferon gamma-related gene in the Indian major carp, rohu (*Labeo rohita*). *DNA Cell Biol.,* **34:** 367-378. https://doi.org/10.1089/dna.2014.2656

Takatsu, M., Uyeno, S., Komura, J.I., Watanabe, M. and

Ono, T., 1999. Age-dependent alterations in mRNA level and promoter methylation of collagen α1 (I) gene in human periodontal ligament. *Mech. Ageing Dev.,* **110:** 37-48. https://doi.org/10.1016/S0047-6374(99)00041-X

Tang, J. and Saito, T., 2015. Effect of type I collagen derived from tilapia scale on odontoblast-like cells. *Tissue Eng. Regenerat. Med.,* **12:** 231-238. https://doi.org/10.1007/s13770-014-0114-8

Thiele, B.J., Doller, A., Kähne, T., Pregla, R., Hetzer, R. and Regitz-Zagrosek, V., 2004. RNA-binding proteins heterogeneous nuclear ribonucleoprotein A1, E1, and K are involved in post-transcriptional control of collagen I and III synthesis. *Circ. Res.,* **95:** 1058-1066. https://doi.org/10.1161/01.RES.0000149166.33833.08

Wang, B., Wang, Y.M., Chi, C.F., Luo, H.Y., Deng, S.G. and Ma, J.Y., 2013. Isolation and characterization of collagen and antioxidant collagen peptides from scales of croceine croaker (*Pseudosciaena crocea*). *Mar. Drugs,* **11:** 4641-4661. https://doi.org/10.3390/md11114641

Wang, L., An, X., Yang, F., Xin, Z., Zhao, L. and Hu, Q., 2008. Isolation and characterisation of collagens from the skin, scale and bone of deep-sea redfish (*Sebastes mentella*). *Fd. Chem.,* **108:** 616-623. https://doi.org/10.1016/j.foodchem.2007.11.017

Xia, W., Hammerberg, C., Li, Y., He, T., Quan, T., Voorhees, J.J. and Fisher, G.J., 2013. Expression of catalytically active matrix metalloproteinase-1 in dermal fibroblasts induces collagen fragmentation and functional alterations that resemble aged human skin. *Aging Cell,* **12:** 661-671. https://doi.org/10.1111/acel.12089

Xu, L., Dong, W., Zhao, J. and Xu, Y., 2015. Effect of marine collagen peptides on physiological and neurobehavioral development of male rats with perinatal asphyxia. *Mar. Drugs,* **13:** 3653-3671. https://doi.org/10.3390/md13063653

Yamada, S., Yamamoto, K., Ikeda, T., Yanagiguchi, K. and Hayashi, Y., 2014. Potency of fish collagen as a scaffold for regenerative medicine. *BioMed. Res. Int.,* **2014**. https://doi.org/10.1155/2014/302932

Yang, C.G., Wang, X.L., Tian, J., Liu, W., Wu, F., Jiang, M. and Wen, H., 2013. Evaluation of reference genes for quantitative real-time RT-PCR analysis of gene expression in Nile tilapia (*Oreochromis niloticus*). *Gene,* **527:** 183-192. https://doi.org/10.1016/j.gene.2013.06.013

Yuan, J.S., Reed, A., Chen, F. and Stewart, C.N., 2006. Statistical analysis of real-time PCR data. *BMC Bioinf.,* **7:** 1-12. https://doi.org/10.1186/1471-2105-7-85

Zarini, A.N., 2016. *Using fruit enzymes for extraction and characterisation of collagen from black Tilapia* (Oreochromis niloticus) *skin.* Universiti Sains Malaysia.