Pathomorphological alterations due to pulmonary hypertension syndrome in broiler chicken reared under temperate climatic conditions of Northern Himalayas

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Abstract

Present study was aimed to investigate path anatomical alterations in commercial broiler chickens with spontaneous Pulmonary Hypertension Syndrome (Ascites). Gross lesions included ascites, cyanotic muscle, right ventricular hypertrophy/ cardiomegaly, and congestion, haemorrhages, and oedema of different organs. Histopathologically marked changes were observed in heart, lungs, liver, kidneys, brain, GIT thyroid, adrenal gland and lymphoid organs. The lesions, in general, included varying degrees of congestion, haemorrhages and perivascular leakage of lymph/oedema; thickening of vascular walls with medial hypertrophy and cystic dilatation of lymphatics; inflammatory changes with predominantly perivascular mononuclear cell and also heterophil infiltration in; neuronal and purkinje cell degeneration and lymphoid depletion. It was concluded that PHS caused marked patho-anatomical changes in all organs. The inflammatory changes observed suggest it as a model for PAH in humans.

Keywords: Broiler chicken, pathology, pulmonary hypertension syndrome

Introduction

Ascites/ pulmonary hypertension syndrome (PHS) syndrome in fast growing broilers is the terminal consequence of progressive pulmonary arterial hypertension leading to cardiac decompensation. ( Wideman et al., 2007) [27]. Cardiomegaly associated with right ventricular hypertrophy and dilatation has been reported consistent feature in PHS (Moayyedian et al., 2011; Davis et al., 2012) [26; 5]. In fact anatomical changes in heart can be detected before a bird exhibits gross ascites syndrome. It is generally accepted that RV/TV index greater than 0.30 is indicative of right ventricular hypertrophy, pulmonary hypertension and, ultimately, ascites syndrome (Wideman et al., 1998) [45]. The syndrome is multifactorial involving complete epidemiological triad including nutritional, management, environmental, and genetic influences (Baghbanzadeh and Decuyper, 2008; Wideman et al., 2013) [1; 44]. Cold exposure and reduced oxygen tension are two important factors favouring development and progression of the disease condition (Julain, 2005; Hassanzadeh et al., 2008) [6; 1]. Owing to high altitude and temperate climate in Kashmir valley, the birds in closed houses frequently encounter the conditions like cold and reduced oxygen tension, and hence are prone to develop ascites. However, many intrinsic and extrinsic factors have been incriminated, warranting detailed path biological studies in given environmental conditions (Hassanzadeh, 2009) [13]. Present study was aimed to investigate detailed pathology of Pulmonary Hypertension Syndrome in broiler chicken.

Materials and Methods

Samples comprised of mortalities from various poultry farms operating in Srinagar, Ganderbal, and Budgam, Districts along with their adjoining areas brought to Division of Veterinary Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir, for post-mortem examination during April 2014 to June 2015. Pulmonary hypertension syndrome was diagnosed on the basis of presence of ascites or right ventricular hypertrophy. The carcasses were subjected to a thorough systematic necropsy examining and gross lesions were
recorded. Also, total ventricular weight and right ventricular weights were taken for 14 cases each PHS/ascitic chicken and normal healthy chicken. Representative samples of liver, heart, caeca, lung, kidney, intestines, bursa, spleen, thymus, thyroid, brain, proventriculus, pancreas, caeca and intestines were collected from ascetic birds, subsequently preserved and fixed in 10% buffered formalin for histopathological examination and processed by routine paraffin embedding technique employing alcohol and acetone as dehydrating agent, chloroform as clearing agent and paraffin wax of congeiling temperature 60 °C. The sections of 5μm thickness were cut and stained with Harris haematoxylin and eosin technique for routine examination (Luna, 1968) [23].

Results
The carcasses revealed varying degree of abdominal distension due to ascites. In high grade ascites abdominal musculature was thin and transparent. The colour of breast musculature varied from pale to dark/cyanotic. Haemorrhages ranging from ecchymosis to suffusions were occasionally observed in thigh musculature. Transudation into coelomic cavity varied from slight quantity of the fluid in caudal coelomic cavity to large quantities occupying all chambers of coelomic cavity. In most of the cases, fluid was coagulated forming gelatinous masses, loose or adhering to viscera (Fig. 1).

Cardiovascular System
Grossly normal to increased quantities of pericardial fluid was observed. Heart invariably contained blood clot in both ventricles and atria (Fig 2a). Other changes included right ventricular hypertrophy with firm consistency of myocardium (Fig 2b) which was frequently associated with dilation of right ventricle, right atrium, sinus venosus, and cardiac vena cava, leading to cardiomegaly (Fig 2c). The mean total ventricular weight (Tv), right ventricular weight (Rv), and Rvw: Tv ratio in normal and ascitic birds were 5.4423 ± 0.30419 vs 5.7229 ± 0.40899, 1.5146 ± 0.09676 vs 1.9562 ± 0.12555 and 0.2775 ± 0.00515 vs 0.3440 ± 0.00456 respectively. In general these weights were significantly higher in ascitic cases (Table 1). Occasionally haemorrhages ranging from petechiae to suffusions were noted on pericardium. In cases complicated with colibacillosis fibrinous pericarditis was noted (Fig 2d). In general blood vessels including cutaneous vessels, major blood vessels in the coelome, and mesenteric vessels were distended with blood (Fig 3).

Table 1: Total Ventricular Weight (Tv), Right Ventricular Weight (Rv) and Rvw: Tv ratio of healthy and PHS affected broiler chicken (Mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Tv (gm)</th>
<th>Rv (gm)</th>
<th>Rvw: Tv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>14</td>
<td>5.4423 ± 0.30419 (4.10 - 8.78)</td>
<td>1.5146 ± 0.09676 (1.02 - 2.57)</td>
<td>0.2775 ± 0.00515 (0.24 - 0.30)</td>
</tr>
<tr>
<td>Ascitic</td>
<td>14</td>
<td>5.7229 ± 0.40899 (4.21 - 8.78)</td>
<td>1.9562 ± 0.12555 (1.49 - 2.87)</td>
<td>0.3440 ± 0.00456 (0.32 - 0.38)</td>
</tr>
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Values in braces are range

Histopathologically, heart revealed mild to severe vascular congestion, focal to diffuse areas of haemorrhage in epicardium and myocardium, and varying degrees of myocardial degeneration. Focal to diffuse mononuclear and heterophil infiltration was observed in myocardium, epicardium and pericardium. Heterophils predominated in birds dead during 3rd week whereas mononuclear were predominant cell type in birds dying during later stage (Fig 4a-c). Occasionally, oedema was observed in birds with mild ascites. In birds with moderate to severe ascites vascular wall was frequently thickened with lateral cystic dilatation of lymphatics and leakage of lymph into the surrounding interstitial space (Fig 4d).

Lungs
Grossly lungs revealed varying degrees of congestion, and haemorrhages. In severely affected cases lungs were oedematous and voluminous. Sanguineous fluid oozed from the cut surface (Fig 5a). Occasionally pneumonia consolidation was observed. Trachea and bronchi were full of dirty frothy exudate. In some cases, caseous plug was present in the trachea. In general, air sacs were clear. Histopathologically, lungs showed moderate to severe congestion of interstitial vessels and blood capillaries, varying degrees of haemorrhages and interstitial oedema. Pulmonary arterioles appeared thickened with medial cystic dilatations. Frequently perivascular leukocytic infiltration, consisting of heterophils admixed with mononuclear cells, was observed. Occasionally plexiform lesions were observed (Fig 5b). In some cases par bronchi, air spaces and air capillaries appeared normal, but frequently broncho interstitial pneumonia and haemorrhagic bronchopneumonia were seen. Broncho interstitial pneumonia was characterized by thickening of interstitium with heterophilic infiltration admixed with mononuclear cells and presence of inflammatory exudates in parabronchi, (Fig 5c). Haemorrhagic bronchopneumonia was associated with presence of necrotic material along with haemorrhagic inflammatory exudates including heterophils and mononuclear cells in the lumen of parabronchi, mesobronchi, and metabronchi, proliferation of fibrous connective tissue in the interstitium, and thickening of the air space wall due to hypertrophy of muscle trabiculae were evident (Fig 5d). Occasionally, osseous metaplasia was noted.

Liver
Grossly, liver was swollen with varying degrees of congestion, oedema and haemorrhages. Frequently the liver surface was smooth but occasionally liver was irregular giving lobulated appearance. In two cases rupture of liver with large blood clot adhering to ruptured site was seen. Occasionally, fibrinous clots of ascitic fluid covered the liver lobes completely (Fig 6).

Histopathologically, liver revealed mild vascular congestion, hepatic cellular degeneration with increased eosinophilia and vacuolar changes. Occasionally rounding and individualization of hepatocytes and focal necrosis with infiltration of heterophils and mononuclear cells were observed. Necrosis was also seen in the perivascular region. Glisson’s capsule was variably thickened, revealing cystic dilatation of lymphatics and leukocytic infiltration. Focal...
necrosis and focal haemorrhages were noted. Kuffer cell proliferation was noted which was more prominent in subcapsular region. Occasionally hepatocytes revealed eosinophilic granular change. Diffuse mononuclear cell infiltration frequently admixed with heterophils and leakage of lymph from cystic lymphatics were seen (Fig 7). In one outbreak basophilic, intracytoplasmic inclusions were observed.

**Kidneys**

Grossly, kidneys were swollen and oedematous distending out from the bony sockets. Frequently they were congested with dark discolouration. However, occasionally pale to greyish white mottling was noted (Fig 8a, b). Varying degrees of haemorrhages were also seen. Histopathologically, kidneys showed mild to severe vascular congestion, focal to wide spread haemorrhages in cortex and medulla, nephrosis, and occasional capsular thickening. Nephritic changes ranged from swelling of tubular epithelium to denudation of cortical tubular epithelium with formation of hyaline and granular casts in lumen. Occasionally swollen glomeruli with vacuolation podocytes and focal nephritis were seen. Inflammatory reaction was characterized by mononuclear and heterophilic infiltration (Fig 8c, d).

**Brain**

Grossly, brain generally appeared normal. Occasionally mild to severe congestion of meningeal vessels was noted. Histopathologically brain revealed varying degree of vascular congestion in meninges, choroid plexus, cerebrum and cerebellum. In cerebral cortex the space around the microvasculature was dilated. Varying degree of degeneration and necrosis of both granule as well as large and small pyramidal cells was seen. The necrotic cells were shrunken with increased cytoplasmic basophilia, and nuclear pyknosis, karyorhexis and karyolysis associated with dialatation of neuropil, mild satellitosis and neuronophagia (Fig 9a). Neuronal degeneration and necrosis, denoted by nuclear condensation and neuropil dilatation associated with satellitosis and neuronophagia, were also observed in thalamus (Fig 9b). Occasionally marked gliosis was noted (Fig 9c). Medullary tracts revealed mild demyelination (Plate 27B). Cerebellum revealed congestion and marked purkinje cell degeneration (Fig 9d).

**Gastrointestinal tract**

Grossly, proventriculus occasionally revealed oedema and thickening of mucosa. Marked congestion of intestines was a consistent feature. Lumen was filled with semisolid or fluid contents (Fig 10a). Often diffuse haemorrhages were seen in duodenum and jejunum. Frequently mucosa presented a velvety appearance. Microscopically, proventriculus revealed mild to severe vascular congestion and oedema in submucosa and mucosa. Occasionally mononuclear cell and heterophil infiltration was evident in mucosa (Fig 10b). Intestines showed mild to severe congestion and oedema of mucosa which frequently extended to submucosa. Frequently, mucous degeneration of goblet cells and varying degrees of heterophils and mononuclear cell infiltration in lamina propria was seen (Fig 10c, d).

**Thyroid**

Grossly, thyroid was oedematous, congested and swollen. Frequently dark discolouration was observed. Microscopically, thyroid revealed mild to severe vascular congestion in the interstitium and capsule. Thyroid follicles of varying sizes lined with essentially squamous type epithelium were filled with colloid. Occasionally active follicles with cuboidal to columnar epithelium and peripheral vacuolation of colloid were seen (Fig 11a). Occasionally mononuclear cell infiltration was noted especially in the perivascular region.

**Parathyroid Gland**

No gross changes were observed in Parathyroid. Microscopically, parathyroid revealed normal densely packed cords of basophilic cells. Occasionally severe vascular congestion was observed (Fig 11b).

**Adrenal Gland**

No gross or histomorphological alterations were observed in adrenal gland, except for marked vascular congestion, focal haemorrhages in medulla and cortex, and occasional cortical necrosis.

**Pancreas**

No gross changes or histomorphological alterations in either exocrine glands or islets of Langerhans were seen, except occasional vascular congestion (Fig 11c, d).

**Spleen**

Grossly, spleen in most cases was enlarged, congested with varying degrees of haemorrhages. Occasionally severe oedema with mottling was seen (Fig 12a). Microscopically spleen revealed mild to severe congestion, areas of haemorrhage and mild to severe lymphoid depletion. In some cases multifocal necrosis and thickening of vascular wall with lateral cystic dilatations with leakage of lymph into the peripheral interstitum was evident (Fig 12b). Spleenic capsule was thickened. Frequently mononuclear and heterophil infiltration was seen in necrotic areas and capsule.

**Thymus**

Grossly, thymus lobes generally revealed congestion. Frequently oedema and swelling was also seen. Microscopically, thymus revealed varying degrees of vascular congestion and lymphoid depletion. Also, varying degree of oedema and infiltration of heterophils in Hessel’s corpuscles were seen.

**Bursa**

Grossly, bursa of Fabricious was oedematous, swollen with varying degrees of congestion and haemorrhages. Lumen often contained serous exudates. Microscopically, bursa revealed mild vascular congestion, focal haemorrhages, interstitial oedema, varying degrees of lymphoid depletion, and infiltration of heterophils admixed with mononuclear cells was seen in mucosa and submucosa. Degeneration of mucosal epithelium and presence of cystic spaces in the mucosa were consistently seen (Fig 12c, d).

**Discussion**

Dark/ cyanotic discolouration of muscles may be attributed to PHS associated hypoxia (Maxwell et al., 1986) [25]. Ascites has been considered as the most prominent lesion in clinical cases of PHS (Dimple et al., 2005) [26]. It has been attributed to systemic hypotension caused by dilation of major veins stimulating rennin-angiotensin-aldosterone cascade leading to sodium and water retension by kidneys, and hence venous...
congestion, increasing hydrostatic pressure and ascitic transudation (Wideman et al., 2013) [43]. Cardiomegaly associated with right ventricular hypertrophy and dilatation has been consistently reported (Olkowski et al., 2003; Davis et al., 2012) [28, 5]. Mouyedian et al. (2011) [26] reported cardiac enlargement due to dilatation of the right atrium, sinus venosus, cardiac vena cava and right ventricle. In cases of early mortality, transudate in coelomewas not marked and right ventricular hypertrophy was not associated with dilatation/ cardiomegaly. However, increase in RV/TV ratio was consistently noted in subclinical as well as clinical cases. This is consistent with the reports by other workers suggesting RV/TV index greater than 0.30 as indicative of right ventricular hypertrophy, pulmonary hypertension and, ultimately, ascites syndrome (Reza et al., 2008; Tekeli, 2014) [30, 36]. Thright ventricular hypertrophy may be attributed to increased pulmonary arterial resistance (Lorenzoni et al., 2008; Lorenzoni and Wideman, 2008; Wideman et al., 2010; West et al., 2010) [17, 19, 41, 59]. The cardiomegaly due to right ventricular dilatation may be attributed to cardiac decompensation associated with weakening of ventricular muscle (Olkowski, 2007; Lorenzoni et al., 2008; Wideman et al., 2013) [27, 17, 19, 44]. Histopathological alterations observed in heart are in congruence with earlier reports (Sijun et al., 2002; Dimple et al., 2005) [34, 5]. However, Habib-ur-Rehman et al. (1999) [10] reported that heart from ascitic birds did not show any significant lesion except mild congestion. The differences may be attributed to stage and duration of ailment as well as concurrent clinical or subclinical affections. The degenerative changes may be attributed to the consistent high cardiac pressure load on account of increased pulmonary resistance due to limited pulmonary reserves (Wideman et al., 2010; West et al., 2010) [41, 59]. pulmonary arterial-constriction (Zhou et al., 2008; Hassan pour et al., 2010) [12, 46], increased flow of blood to lungs under metabolic demand (Lorenzoni et al., 2008) [17, 19] and increased blood viscosity due to elevated haematocrit (Wideman et al., 2013) [44], as well as increased volume load on account of systemic hypotension leading to increased venous return (Wideman et al., 2007, 2013) [42, 44], besides the direct effect of hypoxaemia. Congestion, haemorrhage and oedema of lungs observed grossly and in histopathological sections, are consistent with earlier reports (Maxwell et al., 1986; Biswas et al., 1995; Habib-ur-Rehman et al., 1999) [25, 4, 10]. Lungs besides being the primary target of PHS, have reduced proportionate weight in fast growing chicken when compared with slow growing lines which acts as a predisposing factor for development of pulmonary arterial hypertension (Malan, et al., 2003) [24]. Higher pulmonary arterial pressures and pulmonary vascular resistances with or without differences in cardiac output has been observed to be a consistent feature in PHS susceptible broiler chicken (Lorenzoni et al., 2008; Kluess et al., 2012) [17, 19, 16]. Histopathological lesions in lungs are consistent with earlier reports (Biswas et al., 1995; Habib-ur-Rehman et al., 1999; Dimple et al., 2005) [4, 10, 6]. Medial hypertrophy and intimal proliferation of pulmonary arteries has been consistently reported in broilers with PHS/PAH but is unevenly distributed (Wideman and Hamal, 2011; Bautista-Ortega and Ruiz-Feria, 2012; Weidleer et al., 2012) [43, 2, 38]. Plexiform lesions have been observed in PAH-susceptible lines as early as first week post hatch. The lesions develop from an early compact lesion having relatively homogeneous endothelial matrix and sparse vascular channels into larger mature plexiform lesions exhibiting numerous vascular channels and multiple cell types including connective tissue, inflammatory cells, and proliferating intimal cells (Wideman et al., 2011; Wideman and Hamal, 2011; Kluess et al., 2012) [43, 10]. Immunohistochemical studies have confirmed that the plexiform lesions of broilers contain immune/inflammatory cells viz. monocytes/macrophages, cytotoxic lymphocytes, B cells, and MHC class II cells; and express antiproliferative factors von Willebrand factor, a smooth muscle actin, vascular endothelial growth factor and its type 2 receptor, hypoxia inducible factor-1a, survivin, and tenascin (Hamal et al., 2012) [11]. Predominance of heterophils in some cases may be attributed to concurrent subclinical infections. Varying degrees of liver lesions have been reported in chicken affected with PHS depending on degree of ascites and associated complications (Habib-ur-Rehman et al., 1999; Dimple et al., 2005; Davis et al., 2012) [10, 6, 5]. Histopathological alterations in liver were similar to the changes reported by other workers (Dimple et al., 2005; Davis et al., 2012) [6, 5]. Degenerative and necrotic changes may be attributed to hypoxaemia and venous congestion associated with progressive development of right sided congestive heart failure (Wideman et al., 2013) [44]. Sijun et al. (2002) [34] reported that histomorphologic changes of the lymphatic system of the liver in ascitic broilers were edema, thickening, and cellular proliferation of hepatic capsule; lymph embolism, and lymphatic plasma retention in lymphatic cysts of hepatic capsule. Leukocytic infiltration particularly heterophils and mononuclear cells have been frequently reported (Maxwell et al., 1986; Habib-ur-Rehman et al., 1999) [25, 10]. Dimple et al. (2005) [6] reported that ascitic birds showed acute to chronic perihepatitis along with fibrosis and pseudolobulation in liver. The inflammatory response may be attributed to oxidative stress associated with decreased antioxidants leading to elevated free radicals (Wang et al., 2012) [43]. Grossly kidneys were congested, swollen, oedematous and showed haemorrhages, dark discoulouration and occasionally greyish white mottling. Histopathologically, kidneys showed vascular congestion, haemorrhages, nephrosis, occasional swollen glomeruli with vacuolation podocytes, focal nephritis, and occasional capular thickness. Inflammatory reaction was characterized by mononuclear and heterophilic infiltration. The lesions are consistent with earlier reports (Habib-ur-Rehman et al., 1999; Dimple et al., 2005; Davis et al., 2012) [10, 6, 5]. The renal morphological implications of PHS may be attributed to hypoxaemia leading to increased systemic blood flow, oxidative stress, and metabolic acidosis (Wideman et al., 2013) [44]. Kidneys being the vital organs playing central role in homeostasis and maintenance of blood pressure, the progressive right side congestive heart failure has severe implications (Lorenzoni et al., 2008; Wideman et al., 2013) [17, 19, 44]. The systemic hypotension caused by dilation of major veins stimulates rennin-angiotensin-aldosterone cascade causing sodium and water retention by kidneys leading to vascular congestion further aggravating hypoxaemia (Wideman et al., 2013) [44]. Inflammatory changes may be attributed partially to oxidative stress and concurrent infections. The changes observed in brain may be attributed to consistently aggravating hypoxaemic condition during the pathogenesis of ascites. Ascites in broiler chicken has been invariably associated with increase in blood PCO₂ and decrease in blood PO₂, pH and saturated PO₂ (Malan et al., 2003; Moayyedian et al., 2011) [24, 26]. Scheele et al. (2003)}
Hypoxaemia due to compromised pulmonary
- Malan
- uted generalized vascular response to a cascade
- interferon
- )
- 2014
- extended lymphatic vessels; leakage
- IL
- y be attributed to
- thoracic duct and lymph
- sed by the observation of high incidence of
- increased haematopoiesis and systemic arteriolar dilation
- blood oxygenation incites compensat
- development of PAH and ascites (Lorenzoni and Wideman, 2013) [17, 19, 44]. Stress hormone response also plays a critical role in haemodynamic changes (Lorenzoni and Ruiz-Feria, 2006; Gomez et al., 2007; Zhou et al., 2008; Ruiz-Feria, 2009; Bautista-Ortega and Ruiz-Feria, 2010; Hassanpour et al., 2010; Kluess et al., 2012) [18, 8, 46, 32, 3, 12, 16].

Frequently thickened of vascular walls with medial hypertrophy and cystic dilatation of lymphatics was observed in different organs. et al. (2002) [34] also reported presence of lymphatic cysts bilaterally along the posterior vena cava; protuberances of distended lymphatic vessels; leakage of lymph from the lymphatic cysts into the surrounding; swelling and degeneration of endothelial cells of the thoracic duct; occasionally extensive endothelial cell loss and their exfoliation; and marked dilatation of thoracic duct and lymph embolism, leaking of lymph, edema in some fibers and the enlargement of spaces between fibers, swollen intima, and rupture and bleeding of the thoracic duct. The authors reported significant difference in long and short semi-axis, and the cross sectional area of the thoracic duct, between normal and ascitic broilers. They also reported lymphatic plasma retention in lymphatic cysts of hepatic capsule.

During present study inflammatory changes characterized by predominantly perivascular mononuclear cell infiltrations and also heterophil infiltration, were seen in all organs. Immune system and Inflammation especially perivascular mononuclear cell infiltration including macrophages, dendritic cells, T and B lymphocytes, and mast cells, has been reported to play an important role in pathogenesis of PAH in humans. Expression of inflammatory cytokines such as tumor necrosis factor-α, interleukin (IL)-1, IL-6, chemokines (RANTES/CCL5, CX3CL1/Fractalkine CCl2), T helper cell-type 1 cytokines (i.e., interferon-γ; IFN-γ), and T helper cell-type 1-associated activities down-stream of IFN-γ, such as IL-18 and chemokine CXCL10 has been implicated especially IL-18 and CXCL10 (Kherbeck et al., 2011; Price et al., 2012; Ross et al., 2012; Stacher et al., 2012) [15, 29, 31, 35]. Elevated circulating levels of inflammatory cytokines (IL-1, IL-6) and chemokines (IL-8) as well as C-reactive protein have been associated with local pulmonary inflammatory activity and implicated for the worse clinical outcome in human (Dorfmüller et al., 2003; Kherbeck et al., 2011) [15]. Thus aberrant innate and adaptive immune response including autoimmunity, besides other factors, seems to play a critical role in pathogenesis of PAH in humans (Wideman et al., 2013) [44]. However, characterization of such responses in chicken is warranted.

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