

# Histochemical study of the human umbilical cord in correlation with coiling index

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**Abstract:**

**Introduction:** The umbilical cord is the lifeline between the fetus and the placenta of the mother. It functions throughout pregnancy to protect the vessels that travel between the fetus and the placenta. Histological & histochemical changes of the umbilical cord can have serious deleterious effects on the health of the fetus and newborn. Macroscopic and microscopic integrity of the umbilical cord is vital for its proper functions.

**Materials& methods:** Sample of seventy five umbilical cords with inclusion criteria (full term newborns) whose mothers were (normal) collected from labor room of (Al-Kadhmiya teaching hospital). These umbilical cords were examined as follows:

- Macroscopically (length and umbilical coiling index) and the cords divided into three groups according to the coiling index into (hyper, hypo and normocoiled groups).
- Histochemically (by use of four types of lectin).

**Results:** Lectin histochemistry showed variability in the strength of the lectin binding in different regions of the umbilical cord connective tissue with different coiling indices.

**Conclusion:** The variability in the (umbilical coiling index) was found to be reflected on the histochemistry of the umbilical cord and probably may have effects on the perinatal outcome of the newborn.

**Key words:** Umbilical cord, umbilical coiling index, (hypercoiled, hypocoiled & normocoiled cords) & lectins.

**Introduction:**

The umbilical cord is the vital life line between the fetus and the placenta as it supplies the fetus with the necessary water, oxygen, hormones and nutrients. Therefore umbilical cord abnormalities can lead to a considerable fetal morbidity and mortality<sup>(1)</sup>.

Umbilical cord is a helical (coiled) structure composed of three blood vessels (two arteries and one vein) surrounded by Wharton's jelly (mucous connective tissue) and encased by the strong cover of amniotic epithelium which is a type of squamous epithelium<sup>(2)</sup>. This unique lifeline therefore needs optimal protection, provided by coiling of the umbilical cord & Wharton's jelly which is a specialized tissue acting as a supportive and protective structure substituting for the adventitia of the umbilical vessels<sup>(3)</sup>.

Wharton's jelly consist from stromal cells mainly fibroblast with occasional mast cells and extracellular matrix formed by collagen skeleton and proteoglycans which have a protein core with carbohydrate<sup>(4, 5)</sup>.

Coiling within a certain range (a window of optimal coiling) makes the umbilical cord flexible and strong at the same time, provide resistance to external forces that compromise blood flow, withstand kinking, compression and torsion, which can be demonstrated in a telephone receiver cord<sup>(6)</sup>.

Several researches done previously showed & confirmed that abnormal umbilical coiling index (Hypercoiled & Hypocoiled cords) were associated with increased (adverse perinatal outcomes) like fetal distress, meconium staining, fetal heart rate abnormality, intrauterine growth retardation & intrauterine death<sup>(7-11)</sup>.

Lectins are carbohydrate binding proteins of a non immune origin that agglutinate cells and/or precipitate glycoconjugate. The lectins have no enzymatic activity, may be soluble or membrane bound, and are of bacterial or plant origin<sup>(12)</sup>.

There are various biological roles of lectins in plants, bacteria, and animals<sup>(13, 14)</sup>. At the level of the tissues, lectins have been used in many directions:

1. As specific probes for various cell types<sup>(15)</sup>.
2. Lectins are used to define cells at various stages of differentiation or maturation<sup>(16)</sup>.
3. Lectins are also used as probes to detect microenvironment<sup>(17)</sup>.
4. Functional changes have also been explored by lectins binding<sup>(18)</sup>.

### **Materials&Methods:**

Across sectional study, done in the period between (April, 2010 and August, 2011) in Al-Nahrain College of medicine in the department of human anatomy.

**Collection of the samples:** Samples of the umbilical cords were collected from the labor rooms in (Al-Kadhmiya teaching hospital) With criteria of full term newborns (38-42 weeks of gestation) whose mothers were normal (had no hypertension, no diabetes mellitus, non smoker and had no any other major health problems).

Each of the umbilical cords collected was examined grossly for it's length and the umbilical coiling index. The total number of the umbilical cord used was (75). Measurement of the length done by tape measure with consideration of the umbilical stump of the cord that remains attached to the umbilicus of the baby. The umbilical coiling index was determined by dividing the total number of the coils by the total length of the cord in centimeter<sup>(2)</sup>. An umbilical coiling index of less than (0.17) and more than (0.37) was accepted as being hypocoiled and hypercoiled respectively, between (0.17 and 0.37) was normocoiled<sup>(1)</sup>.

**Preparation of the paraffin sections:** The umbilical cord tissues parts were selected from the proximal, middle, and distal segments of the cords, these parts were prepared for paraffin sections as follows (Fixation, dehydration, clearing, infiltration, embedding, sectioning, dewaxing, hydration, staining & mounting)<sup>(19,20)</sup>.

Small parts of the umbilical cords were taken by cutting transversely by a sharp knife across the cord. Pieces fixed with 10% formalin. Dehydration done by transferring the samples into graded concentration of ethyl alcohol. Clearing done by transferring the specimens into paths of xylene. Infiltration and blocking by electric wax dispenser used for embedding the sections in a labeled baths of a molten paraffin wax with melting point of 58 c°. Then the specimens were blocked in paraffin wax using embedding moulds (L-shaped). The paraffin blocks were kept in refrigerator at 4 c° until used. Sectioning using the electric microtome Serial sections of 8µm thickness were cut, these sections (about 3-5 sections per slide) were transferred to a hot water bath with a temperature of 50 c° before attaching the sections to the slides. Dewaxing and hydration of the sections with xylene followed by passing the slides through graded less concentrations of ethyl alcohol baths then the slides were transferred to be hydrated in distilled water.

**Procedure of lectin histochemistry:** All lectins used were obtained from sigma. They were flourescein isothiocyanate (FITC) labeled. (Table-1) shows the lectins used in this study and their carbohydrates specificity.

**Table-1:** lectins used and their specifications.

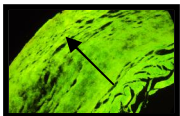
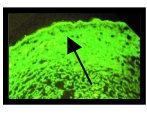
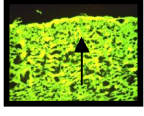
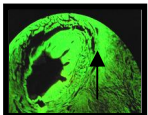
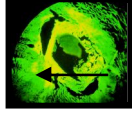
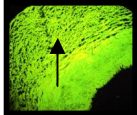
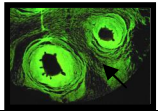
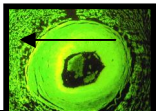
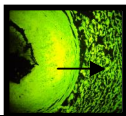
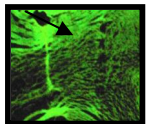
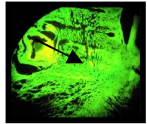
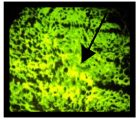
(Lectin Common name), Abbreviation	Saccharide specificity	Lectin Concentration
(wheat Germ Agglutinin) <b>WGA</b>	Nacetylglucosamine ( 1,4GlcNac) Acetylneuraminic (sialic acid)	200µg/ml
(SuccinylatedWGA) <b>SWGA</b>	Nacetylglucosamine ( 1,4GlcNac)	200µg/ml
(Soya Bean) <b>SBA</b>	-& Nacetylglactosamine. -& Galactose.	200µg/ml
(Concanavalin Agglutinin) <b>CON-A</b>	Mannose	200µg/ml

The Procedure of lectin histochemical staining as follows:

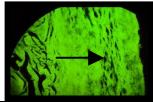
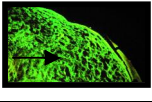
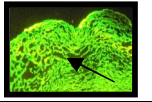
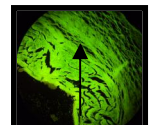
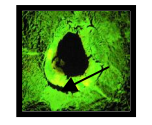
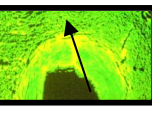
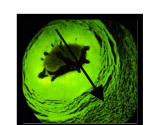
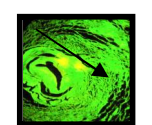
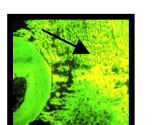
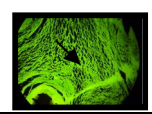
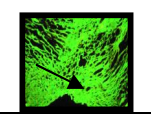
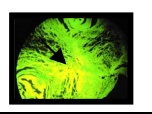
- Dewaxing of the paraffin sections was done by use of xylene for 30 minutes.
- Hydration of the sections through graded concentrations of ethyl alcohol.
- Hydrated paraffin sections were washed in phosphate buffered saline (PBS).
- Keep the slides flooded by the lectin-PBS solution.
- Sections were flooded by lectin-PBS solution and kept for 1.5 hours..
- Sections were washed in PBS and mounted in non-fluorescent fractoil mountant.
- Sections were examined under the u/v light of the fluorescent microscope.
- Digital camera was used for capturing pictures.

### **Results:**

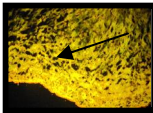
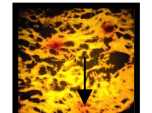
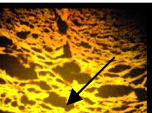
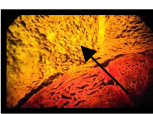
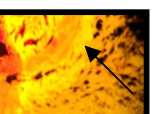
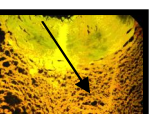
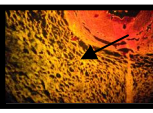
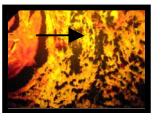
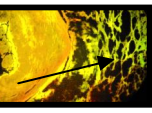
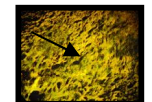
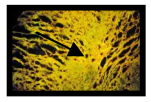
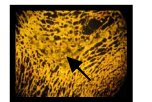
SWGA, WGA, SBA& CON-A Binding in different regions of the umbilical cord connective tissue (subamniotic, perivenous, periarterial & central region) showed Variation with the degree of the (umbilical coiling index) as shown in the following figures:

	Hypercoiled cords	Normocoiled cords	Hypocoiled cords	Variation with coiling
<b>1.subamnion region</b>				<b>Decreased binding</b>
<b>2.perivenous region</b>				<b>Increased binding</b>
<b>3.periarterial region</b>				<b>Increased binding</b>
<b>4.central region</b>				<b>no variation in binding</b>

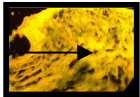
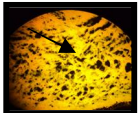
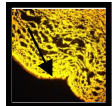


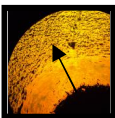
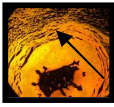
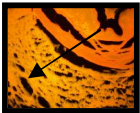
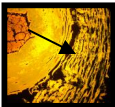
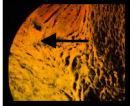

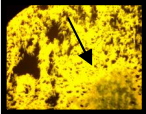
**Figure-1:** (SWGA) binding to different regions of the umbilical cord (at heads of arrows) and it's variation with coiling (X40).

	Hypercoiled cords	Normocoiled cords	Hypocoiled cords	Variation with coiling
1.subamniotic region				Increased binding
2.perivenous region				Increased binding
3.periarterial region				Increased binding
4.central region				no variation in binding

**Figure-2:** (WGA) binding to different regions of the umbilical cord (at heads of arrows) and it's variation with coiling (X40).

	Hypercoiled cords	Normocoiled cords	Hypocoiled cords	Variation with coiling
1.Subamniotic region				No variation In binding
2.Perivenous region				Decreased binding
3.Periarterial region				No variation In binding
4.Central region				Decreased binding

**Figure-3:** (CON-A) binding to different regions of the umbilical cord (at heads of arrows) and it's variation with coiling (X40).

	Hypercoiled cords	Normocoiled cords	Hypocoiled cords	Variation with coiling
<b>1.Subamniotic region</b>				<b>No variation in binding</b>
<b>2.Perivenous region</b>				<b>Decreased binding</b>
<b>3.Periarterial region</b>				<b>No variation In Binding</b>
<b>4.Central region</b>				<b>Decreased binding</b>

**Figure-4:** (SBA) binding to different regions of the umbilical cord (at heads of arrows) and it's variation with coiling (X40).

### Discussion

The concluded comparable regional strength in each of the three variable coiling indices was used as a parameter to compare the variability of the strength of fluorescent lectin binding during the increasing coiling index of the cords. The system of comparable evaluation of the fluorescent lectin binding was applied in most of the literatures involving lectin binding as a tool to detect microenvironments<sup>(15, 17&21)</sup>.

It seems that the beginning of umbilical cord coiling (the hypocoiled cord) results in condensation of the loose connective tissues at the perivascular regions, this condensation is associated with a abundance of intercellular N-acetyl-glucosamine (marked by increasing WGA & SWGA binding with coiling). The perivascular connective tissue maintained overflowing of N-acetyl-glucosamine during hypercoiling.

The slight coiling (the hypocoiled) subamnion connective tissue is also condensed and is marked by the same sugar. This conclusion is verified as strong SWGA & WGA binding at the subamnion and the perivascular tissues in the hypocoiled cords.

In advanced stages of cord coiling (hypercoiled cord), the sialic acid prominently represented at the subamnion, this is marked by the comparable decreasing SWGA binding and comparable increasing WGA binding with coiling. The lectin SWGA binds to N-acetylglucosamine, while the lectin WGA bind to N-acetylglucosamine and sialic acid.

The increase of some glycoconjugates carbohydrates and the loss of others in the umbilical cords from pregnancies with minor degree of glucose intolerance had been related to it's morphofunctional alterations<sup>(22)</sup>. Therefore the different strength of lectin regional variation in this study may be related to different regional morphofunctional changes in the connective tissue glycoconjugates that associated with the degree of coiling of the umbilical cord.

The lectins were used as a specific probes to indicate various cell types<sup>(15)</sup> and used to define cells at various stages of differentiation or maturation<sup>(16)</sup> that probably explain that the different types of lectin binding in different regions of the umbilical cords in this study is possibly related to different nature of cells. The difference in the nature of these cells may be related to different preexisting formation, this interpretation was based on the observations made by Fine structural, immunohistochemical<sup>(23, 24 & 25)</sup> and in vitro functional studies<sup>(26,27)</sup> which showed that there are significant differences in the number and nature of cells among subamniotic, intervacular and perivascular regions. This leads to the hypothesis that those regions might be originating from different pre-existing formations.

A gradual change in morphological and probably functional properties of Wharton's jelly cells during the course of pregnancy was also noted<sup>(24, 28)</sup>.

The N-acetyl-d-glucosamine, sialic, d-Galactose(beta1-->3)-N-acetyl-d-galactosamine, N-acetyl-d-galactosamine were found in normal umbilical cords<sup>(22)</sup>. In this study highlighted the presence of these carbohydrates in different regions of the umbilical cords.

The findings of (ConA, WGA and SBA) binding in the different regions of the hypercoiled, normocoiled and hypocoiled cords is in agreement with reports of. A previous study used a battery of horseradish peroxidase conjugated lectins including (ConA, WGA, and SBA) and revealed that there was a role played by the sugar residues in the normal and pathological umbilical cords<sup>(29)</sup>.

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